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FINAL REPORT

Institution: ⁵⁶ INSTITUTO DE AGROQUIMICA Y
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Patronato "Juan de la Cierva"
Jaime Roig, 11
Valencia, Spain

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Principal Investigator: Dr. Salvador Barber

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Investigations were divided into three areas: (1) Chemical composition of rice; (2) changes during storage of milled rice; and (3) rice quality.

(1) Chemical composition of rice. The distribution of the following chemical constituents in rice was investigated: 1) Carbohydrates: Starch, amylose, amylopectin, total, reducing and non-reducing sugars, and individual sugars. 2) Nitrogen compounds: Total (TN), protein (PN), and non-protein (NPN) nitrogen, protein solubility fractions (PSF), sulfhydryl and disulfide groups, and free amino nitrogen. 3) Enzymes: Alpha-amylase, beta-amylase, proteolytic activity, cysteine desulfhydrase, and cystine reductase. 4) Lipids: Total lipids, lipid fractions (neutral fats, free fatty acids and phospholipids), fatty acid composition of each three fractions, and chemical characteristics of lipids (acid, saponification, ester, iodine, peroxide and TBA values). A concentration gradient was found for all constituents and fractions investigated. Qualitative and quantitative differences between successive layers of the kernel were found, they showing that the actual chemical nature of rice is different from and much more complicated than that indicated by the average chemical composition data, conventionally used up to now. Although varietal differences were found concerning concentration data, the pattern of distribution of chemical constituents was common for all varieties investigated. The outermost layer contains several times more sugars, proteins, free amino acids, free fatty acids, phospholipids, enzymes, etc., and less starch and amylose than the nucleus of the kernel. Knowledge of the outer layer is fundamental to achieve a proper understanding of the reactivity and properties of rice.

(2) Changes during storage of milled rice. Laboratory-scale studies were conducted on rices of different milling degrees (7.6%, 9.8% and 12.6%), held at different conditions of temperature (-20° , $+5^{\circ}$, $+25^{\circ}$, and $+35^{\circ}\text{C}$), moisture contents (12.9 - 13.3%, 14.2 - 14.6% and 15.5 - 15.7%) and atmospheres (hermetic and aerated storage). The effects of storage on organoleptic properties (odor, color, cohesiveness, and overall acceptability), physico-chemical characteristics (water absorption, residual cooking liquids, alkali test, gelatinization and pasting characteristics, N index and intergranular atmosphere) and composition (every constituent and fraction cited above) were determined. Compositional changes were comparatively investigated in the outer layer, the nucleus and the entire kernel. In addition, changes in microflora of milled rice (bacteria, and mold and yeast counts, % mold-infested kernels, and types and proportion of microorganisms) were also studied.

Storage resulted in decreased cohesiveness, dryer surface and firmer texture of cooked kernels; unsafe conditions resulted in darkening and development of off-odors, which counteracted quality improvement. The keeping quality of air-tight stored milled rice as a function of milling degree, moisture content and temperature, was determined and it is given in a diagram. Water absorption at 100°C increased and residual solids decreased. Alkali digestibility remained practically unchanged. Amylogram changes were dependent on storage conditions; N index varied with conditions; in general values increased with time, but decreased after deterioration of rice. CO_2 in intergranular air increased. Starch and amylose contents remained unchanged. RS increased, and NRS decreased; the magnitude of each change varied with conditions. There were no significant changes in TN, PN, and NPN contents. PSF decreased, every one at a different rate dependent on location within the kernel. SH and SS contents decreased under prolonged storage. FAN content and enzyme activities decreased. Undermilling and high moisture content and temperature favoured storage changes.

A major part of total changes in rice occurred in an extremely thin (less than 0.1 mm) outer layer. Separate consideration of this outermost layer affords a more actual and sensitive means than the entire kernel to follow storage changes.

Storage decreased the number of microorganisms and varied their types and proportion. Airtight-storage resulted in increased proportion of *Xantomonas* and *aspergilli*.

(3) Rice quality. Studies directed to determine the role played by major and minor constituents of rice in its processing and cooking behavior were conducted along three lines of research: a) to obtain fundamental data on chemical composition of different rice varieties and correlate them with subjective measurements of rice quality; b) to follow simultaneous changes during storage in composition or physicochemical characteristics and in organoleptic properties, and determine the existence or lack of correlation between both changes; and (c) to prepare artificially treated rices with only one or determined constituents modified, and compare quality and chemical or physicochemical changes in order to ascertain the factor(s) determining the cooking and eating properties of rice.

Results from all three lines indicated that the composition of the outer layer of the rice kernel largely governs cooking and eating quality. The influence of proteins and starch on the microscopical structure and properties of the cooked rice kernel was studied using rices of different quality and age, and rices treated with amylolytic and proteolytic enzymes. The protein material appeared to be the major factor responsible for both rice quality and quality changes during storage. The amount of proteins in outer layer may be a useful criterion to evaluate rice but it is not always sufficient. The uniformity of the protein-rich outer layer, as well as the chemical nature of the protein material should be taken into account. The starch-protein binomial may also be of interest in some cases.

The nature of proteins modification in rice during normal and accelerated aging, and through artificial treatments was investigated. The effects of treatment of rice with breakdown products of lipid oxidation on rice properties and composition were found to be parallel to those of normal and accelerated storage. Role and effects of lipids were studied using defatted, defatted processed, and defatted with methyl linoleate added rices. Results indicated that a protein modification, the nature of which appears to be an inter- or intramolecular crosslinking resulting from a protein-carbonyl compounds interaction, was mainly responsible for changes in rice properties.

Special emphasis was placed on literature survey; 403 references are given.

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INTRODUCTION

Production is the main problem of present rice situation. In 1985 the ever increasing rice demand will probably exceed in 100 million tons the level of 1961-63 (58). Rice will continue being fundamental source of calories and proteins for one half of the world population, just that one of more scanty resources. The basic role of rice in the world supply of food emphasises the importance of improvement of its nutritive value and its optimum utilization.

Quantity and quality of the product destined to human consumption depend largely on the efficiency of technology. Better utilization of bran, higher milling yields and reduction of storage losses are examples of important goals which would alleviate the present situation, particularly in underdeveloped countries. In overdeveloped countries, rice demand in terms of raw product is decreasing. However, the demand for processed products is increasing significantly. Industry needs more and more information on the characteristics and properties of rice in order to use and process more adequately each variety or lot and to obtain an end product of constant and specified quality. Evaluation of rice samples based on objective criteria is needed for the selection of varieties and for marketing.

The possibility of having an advanced technology depends, above all, on the possession of fundamental knowledge on composition, properties and processing behavior of rice. Without this basis, perspectives for development of the rice sector are little promising.

The present project research is intended to afford a partial contribution to this problem as it concerns to the basic knowledge of the composition, properties and behavior of the rice grain.

The primary objective of this investigation was to obtain information on the organoleptic, physicochemical, and biochemical changes in milled rice during storage. Milled rice obtained from freshly harvested paddy differs in its cooking properties, processing characteristics and nutritive qualities from rice which has been previously stored or aged. Little was known about the constituents responsible for such changes, even about the changes themselves. Furthermore, most of the then existing knowledge concerned to rough rice, which behavior might be different. This, and the fact that increasing quantities of milled rice were moving into the international market, made basic research on storage changes in milled rice advisable.

A second purpose of this project was to develop basic knowledge of the interrelationships between chemical constituents and physical characteristics as affecting processing and cooking characteristics. Such study was approached in a

previous project research^(*) in which, after an extensive investigation of the constituents of rice that influence quality, a tentative method for the objective measurement of rice quality was developed. Present study of storage changes was particularly suitable for verifying the general validity of the new test. Furthermore, data on concomitant changes in quality and composition might show new approaches to the quality problem, or even afford basic knowledge to develop new or improved tests for quality.

Information on the distribution of chemical constituents in the rice kernel and their characteristics was needed for basic studies both on aging of milled rice and rice quality. Previous findings^(*) on the heterogenous location of constituents within the kernel, emphasised the need for this knowledge and it became the third objective of present project research.

The present investigations have been divided into three areas:

- I. Basic studies on the chemical composition of rice.
- II. Basic studies on aging of milled rice.
- III. Basic studies on rice quality.

They will be dealt with separately.

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I. BASIC STUDIES ON THE CHEMICAL
COMPOSITION OF RICE.

INTRODUCTION

The average chemical composition has generally been the basis for studying the rice kernel and the effects of technological processes on it. However, the rice kernel is not a homogeneous mixture of carbohydrates, proteins, lipids and minor constituents -minerals, vitamins, enzymes. The kernel is quite a heterogeneous system and consequently, data on its average composition provide only an inaccurate knowledge of its chemical nature. This knowledge is insufficient and, very often, misleading.

It is well known that the degree of milling determines the amount of nutrients in the residual milled kernel. Fundamentally the vitamins and the mineral constituents are present in greater quantities in the germ and the bran than in the endosperm. The realization of this fact palliated malnutrition and deficiency diseases in rice eating areas in the past. However, in 1964, when this project was initiated, the heterogeneous distribution of the chemical constituents of the milled rice grain had not been investigated adequately. It in spite of that such information is indispensable for possessing a real knowledge of rice, and for a better understanding of its properties and behavior during storage and processing.

The work described below is intended to obtain an extensive and detailed knowledge on the distribution of the chemical constituents in the rice kernel, paying particular attention to the milled kernel. The work comprises the study of carbohydrates, nitrogen compounds -including enzymes- and lipids.

LITERATURE REVIEW

- covering up to 1964(×) -

1. PRINCIPAL PARTS OF THE RICE KERNEL.

The rice kernel can conveniently be regarded as consisting of three main parts (××): a) the hull, b) the "total bran", and c) the milled kernel. It has been estimated that on the average a), b) and c) amount respectively 17-21, 9-13 and 70-75 per cent (3) (27) (59).

(×) More recent works are included in the section: Discussion.

(××) For standardised rice terminology see (1) (2) (3) (35),

In practical milling two fractions, bran and polish, may be obtained instead of "total bran", their relative proportion being approximately 4:1. Bran may in turn be roughly fractionated at the mill in rice bran and germ; the latter represents about 2 per cent of milling products (27).

2. GROSS CHEMICAL COMPOSITION OF THE PRINCIPAL PARTS OF THE RICE KERNEL

The approximate composition of main milling fractions of rice is given in Table I. A tabulated summary of the published data has been compiled by Juliano (5). Fig. 1 shows graphically the important differences in composition among these fractions.

3. HISTOLOGICAL COMPOSITION OF THE PRINCIPAL PARTS OF THE RICE KERNEL

A brief review of the morphology of the grain seems pertinent for a better understanding of the distribution of constituents.

3.1. Hulls

"The hull includes the lemma and palea. Structures such as the rachille, sterile lemmas, the awn if present, and broken segments of the pedicel are usually associated with the hull, if they survive the threshing process" (1). Shelling in rubber roll husker—as used in Spain and other countries—removes the hull without ponderable loss of bran. Hull percentage may vary from 16 to 35 percent among varieties (1). Hull in Spanish rices ranges about 18 percent (27).

3.2. Total bran

It is a complex fraction. It includes the pericarp layers, tegmen (x), aleurone layer and embryo. During the process of milling all these fractions, and a small portion of the endosperm are removed as bran (1). Sometimes, bran contains particles of rice hulls that got into it accidentally (13).

The bran layer enveloping the endosperm is "thicker along the longitudinal dorsal line or "ridge" than around the lateral and ventral surface of the kernel" (20) (7); the measurements carried out in 23 rice varieties cultivated in India ranged 34–62 μ and 21–41 μ , respectively (7).

a) The pericarp is light brown, speckled reddish-brown, red or purple (1). It is fibrous and varies in thickness—reported values: 20–60 μ (7) (6). Cell walls are about

(x) According to (1) it is often misnamed "testa".

TABLE 1.- Proximate composition of rice milling fractions.

Constituent	Rice hulls ^(a)	Rice bran ^{(b)(c)}	Milled rice ^(b)
Protein (%)	1.80- 4.40	7.3-19.1	4.5 -14.3
Fat (%)	0.50- 1.34	6.4-26.2	0.04- 4.6
N-free extract (%)	24.7 -32.7	37.2-68.8	82.8 -94.3
Fiber (%)	35.0 -48.3	4.0-36.6	0.1 - 1.0
Ash (%)	15.7 -22.3	6.3-23.7	0.2 - 1.9
Pentosans (%)	16.94-21.95	8.6-16.3	0.4 - 2.2
Moisture content (%)	2.4 -11.0	8.9-14.7	9.4 -13.2

(a) Data taken from (12)(14)(27)(36)(37)(38), adapted for % dry basis.

(b) " " " (12)(5), adapted for % dry basis.

(c) This fraction comprises: true bran, polish and germ.

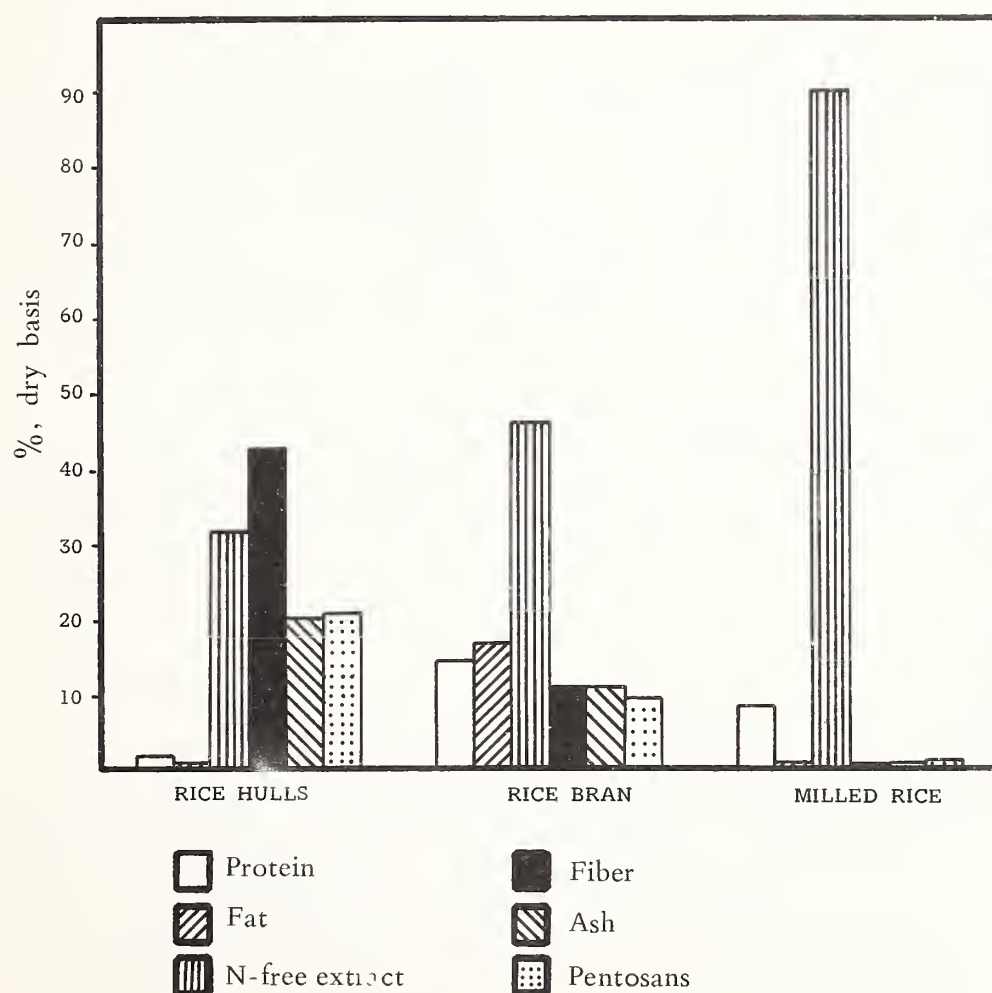


Fig. 1.- Comparative composition of rice milling fractions

2 microns thick (20). "The pericarp layers may be differentiated into epicarp, mesocarp and endocarp". The outermost layer, the epicarp, is unicellular; flattened cells of $3-7 \times 40 \mu$, lay parallel to the major axis of the kernel. Underneath is the mesocarp, composed by two to five cell layers; cells $4-13 \times 183 \mu$ in size are oriented transversally to the major axis of the kernel. Finally, the inner layer, the endocarp -also called "cross layer" (3)- is formed by elongated cells of $60-200 \times 4-10 \mu$ size (6).

b) "Next to the pericarp are two layers of cells representing the remains of the inner integuments, the tegmen or seed coat" (1), of about 6.5μ thick (6).

c) "The aleurone layer lies beneath the tegmen and is enclosing the endosperm. It consists of " quadrangular or rectangular parenchyma cells (7) of $20-30 \times 40 \mu$ in size, with thin walls of about 2μ thick (20). The aleurone layer is not uniform in thickness. It has less cell layers in the ventral -one to three layers- than in the dorsal side -two to six; some reported values for thickness in both sides are $15.3 - 18.0 \mu$ and $26.8 - 43.1 \mu$ respectively (13).

d) "The embryo, of about one third the length of the fruit" (13), lies on the ventral side of the spikelet next to the lemma. The embryo contains the embryonic leaves (plumule) and the embryonic primary root (radicle). The plumule is enclosed by the coleoptile and the radicle ensheathed by the coleorhiza; these form the embryonic axis. The embryonic axis is bounded on the inner side by the scutellum (cotyledon) which lies next to the endosperm. The coleoptile is surrounded by the scutellum and the epiblast, the vascular trace which is fused with the lateral parts of the scutellum (1). A detailed description of morphology of the rice embryo is given by Santos (13).

The proportion of the rice grain that the different parts of bran represent has been determined in fractions obtained by dissection methods: pericarp aleurone 5.95%, covering to germ 0.20%, epiblast 0.27%, coleorhiza 0.2%, plumule 0.31%, radicle 0.17%, scutellum 1.25% (9). Pericarp, aleurone and embryo amount respectively 2.3%, 4.7% and 2.3% of the grain (8).

3.3. Endosperm

"The endosperm is enclosed by the aleurone layer, which lies beneath the tegmen" (1). In the embryo concavity, the outermost layer of the scutellum -known as epithelium (13)- contacts the endosperm. Cell structure of endosperm is not uniform. It shows a pattern of cell arrangement in which a central core of small cells is surrounded by larger cells radially oriented (13) (133) (20). Cells of the center are nearly isodiametric, ranging in size from about 45×50 to 80×105 microns. Length-width ratios of these cells in cross section are approximately 1.0-1.2. No relationship has been demonstrated between the measurements of

central cells and grain length". "Cells immediately lateral to the central core are larger, and either isodiametric, slightly elongated, or slightly flattened radially (estimated ratio 0.7-1.4). Cells adjacent to the aleurone are smaller and many are flattened radially (estimated ratio 0.2-1.0)". "Cells immediately dorsal and ventral to the core are various in size, especially in the radiating dimensions. Ratios of radiating to sectoring dimensions likewise correspond roughly to grain length. Cell areas are at variance with ratios, and show no relation to grain length" (20). "The value of cell dimensions in characterising rice varieties has not been determined" (20). Cells of the outermost layer of the endosperm are isodiametric (size 20-40 μ) (6).

Endosperm cell walls are very thin (about 0.25 microns). The occurrence of a middle lamella and two primary wall layers has been suggested but not established as common (20).

Endosperm is chalky in glutinous rices and totally or partially translucent in non glutinous ones. In the latter a "white chalky region extending to the edge of the ventral side and towards the center of the endosperm is called a white core" (1). Opaque regions, softer and more fragile, do not show a different cell structure but a different arrangement of aggregated starch granules (40). Two parallel ridges on each of the flat surfaces of the kernel line longitudinal grooves which are inconspicuous in some varieties but remarkable in others, then affecting the efficiency of whitening during milling.

In processing the pericarp, seed coats, embryo, most of the aleurone layer, and also a portion of the outer layer of the endosperm cells are removed. Removal is not uniform, depending largely on the geometry of the kernel - particularly on the depth of the grooves cited above. After removal in four successive whitening cones of 6.1 percent of the brown kernel weight, aleurone residues still are present (6) (see also (13)).

4. DISTRIBUTION OF CHEMICAL CONSTITUENTS WITHIN THE RICE KERNEL^(*)

4.1. Carbohydrates

The following carbohydrates have been reported to be present in the dehulled rice grain: starch, dextrans, sugars, hemicelluloses, cellulose, pectic substances and gums. All of them appear to be heterogeneously distributed within the rice kernel. Heterogeneity has been shown by histochemical studies and by chemical analysis of by-products fractions from rice milling.

(*) Hulls are not included here.

4.1.1. Starch. Starch is absent in the pericarp, tegmen and aleurone layers of mature rice (13). It "is present in the pericarp layers in the early stages of development but is exhausted as the grain develops" (39). Recently, a few waxy starch granules have been noted in aleurone cells (40). The embryo contains no starch as shown by histochemical (13) and chemical means (29); being abundant in the early stages of development, it becomes exhausted by harvest time and then only traces of starch have been found in the scutellum (39). (A 2.42%, dry basis, of starch content has been reported for rice germ (26); however, this value was obtained by calculation from oses, holosides, dextrans and total glucides data). In contrast with this, starch is -as it is known- the major component of rice endosperm.

Chemical analysis of successively removed bran fractions show that starch content increases with deepness of milling (10). Data from various authors on carbohydrate content (not including cellulose) of bran from successive whitening cones (14), as well as on starch (41) and nitrogen free extract of bran and polish (12) (5) also show an increasing concentration gradient from outer towards inner regions of the kernel. Starch losses during rice milling amount approximately 5-6 per cent (2) (5) (17) (22). The lack of starch in outer covers -even in the aleurone layer- and the non uniform removal of layers of different deepness during milling, impose some limitation regarding the distribution pattern of starch suggested by these data. Nevertheless, histochemical observations have demonstrated differences of qualitative and quantitative character in starch within the endosperm.

The endosperm cells are filled with starch granules embedded in a proteinaceous matrix. "Diameters of individual starch granules range from 2 to 9 μ and average between 5 and 6 μ , regardless of variety". Starch granules are small (2-4 μ) in the peripheral cells, and larger (5-6 μ) in the major central portion of the kernel; extremely small granules are found in cells near the germ (20). The higher resistance to the action of sodium hypochlorite of starch of the outer layer of rice endosperm as compared with that of the center of the kernel has been ascribed to differences in their chemical properties (6). The rate of starch hydrolysis by diastases has been found to increase with increased polishing; differences have been ascribed to the inhibiting action of bran (23).

No systematic study on the distribution of starch within the rice kernel was published -as far as it is known- before 1964. However, a concentration gradient for starch in the endosperm -at least in its outer layers- is indicated by starch content data for milled rices with progressive milling degrees (15) (16) (19).

4.1.2. Dextrins. Available data on dextrin content of milling fractions are rather scarce. Yusta and Santos (26) reported 3.86% dextrins in rice germ; Soldi (29) found 14.23% dextrins -containing erithrodextrins. In polished rice dextrins amount to 0.85 - 1.06% (57).

4.1.3. Sugars. According to the histochemical work of Santos (13), pericarp, tegmen and aleurone layers possess no soluble carbohydrates, whereas embryo is rich in them. Reported

values for sugar content of rice germ vary widely with the source of data, most probably because of purity of samples. Total sugars amount about 8% (29) (10) or more and levels for reducing sugars range from 1% (10) to 11.63% (26). Total and reducing sugar contents of bran fractions -manually degermed- from successive whitening cones varies with milling degree, showing first an increasing concentration gradient, reaching a peak -before final milling- and then decreasing towards the center of the kernel (10). Comparison of total, reducing and non reducing sugar contents of brown and white rice (42) shows that sugars content of brown rice is twice that of milled rice. The observation by Santos (13), cited above, leads to envisage that such variation might be mainly due to the ratio true bran/endosperm which is affected by the milling degree. Data for different endosperm layers -free of bran- are needed to adequately elucidate whether sugars are or not heterogeneously distributed in milled rice.

Regarding individual sugars, glucose, fructose and sucrose have been reported to be present in brown rice, rice bran, rice embryo and milled rice (42) (43) (46) (47) (30) (29) (48); raffinose, in all except in rice bran; maltose, in all except in rice bran and rice embryo; maltotriose and maltotetraose only in brown rice (47); galactose, isomaltose, arabinose, and xylose only in milled rice (46). The principal non-reducing sugar in both brown and polished rice is sucrose and the reducing sugar portion is almost entirely glucose (42). In rice embryo a similar situation has been found (48), although there is a work (29) reporting that total sugars contain 34.38% glucose, 21.53% fructose and 14.5% sucrose.

4.1.4. Cellulose. Cell walls of the pericarp, aleurone layer and endosperm react positively to the zinc-chloride-iodine reagent, suggesting the presence of cellulose (20). Distribution of cellulose within the kernel is heterogeneous: bran is rich in cellulose, values ranging from 8% to 18% (5) (41), rice polish is poorer (1.1% - 7.7% (5) (41)), and rice germ is intermediate (3.4% - 10.5% (10) (26) (41)); fiber content of milled rice is less than 1%. Fiber content of bran fractions -free of entire germ- from successive whitening cones decreases with milling degree (10) (14).

Kihara et al. (56) did not find any significant difference in the alpha:beta:gamma celluloses ratio between rice bran and rice.

4.1.5. Hemicelluloses and other carbohydrates. Occurrence of hemicelluloses in cell walls of the pericarp, aleurone layer and endosperm has been shown by histochemical tests (20). Differences among the various milling fractions are known: bran is rich in pentosans -values range from 8.6% to 16.3% (5), 6.0% for hemicellulose (18)-, rice polish is poorer -0.1-5.0% (5)-, and rice germ is intermediate -7.4% (26)-; pentosans content of milled rice is about 1.5-2.0% (5) although lower values have been reported (54) (60) (18).

Hemicelluloses B of bran and milled rice appear to be similar in total reducing sugars content (67.9% and 64.7% respectively), however, the former contain

more pentoses (59.6%) than the latter (43.1%) (18). Xylose, arabinose and uronic acid were found in both hemicelluloses, but galactose was only detected in bran while glucose and aldobiuronic acid only in polished rice (18). Xylose and arabinose are the components predominant in amount in hemicellulose (50) (18). It may be of interest to note that manose has not been detected in hemicellulose (18).

Four higher oligosaccharides have been reported to be present in brown rice (47). Gums have been reported in bran like in polished rice, they being more abundant in the former -2.9% (61)- than in the latter -0.8/1.9%, including sugars (62).

4.2. Nitrogen compounds

There is evidence for a heterogeneous distribution of nitrogen compounds in the rice kernel. Proteins, the most well studied fraction, is known to occur in quite different concentrations in the various anatomical parts of the kernel, milling fractions and even within an anatomical part.

4.2.1. Proteins. According to Santos (13), pericarp and tegmen possess no proteins; however, Little and Dawson (20), in a more recent histochemical work, found that "cell walls of the pericarp reacted positively to the Erithrosin B and ferric-ferricyanide tests for proteins". Aleurone is rich in proteins (13) (22). The embryo too (13). Proteins in the latter are known to be heterogeneously distributed; they are mainly concentrated in the parenchyma cells of the plumule and the pericarp root, together with the corresponding epithelial cells, as well as in epithelium of the scutellum, inner cells of the cotyledon and epiblast (13). It has been reported (7) that "the proteins are first concentrated in aleurone layers and later (seven days and more after fertilization) they pass on to the adjacent endosperm cells and those parts of the embryo which are nearer to the aleurone layer". The cited authors (7) in investigating the proteins in the rice grain and the development of the aleurone layer found that "one or two layers of the cells of the endosperm adjacent to the aleurone layer are rich in protein" (7). Borasio in 1929 (22) and Santos in 1933 (13) found similar results. In a more recent work (20), published in 1960, such histochemical information has been confirmed. It is of interest to see that, as quoted by Ramiah and Mudaliar (7), the occurrence of large quantities of proteins in the peripheral layers of the endosperm was found in wheat by O'Brein in 1895. The work of Little and Dawson (20) showed that "protein was most concentrated in peripheral-lateral and peripheral-ventral cells where starch granules were fewest, was least concentrated in peripheral-dorsal cells, and was often rather sparse in central, dorsal and ventral cells". Protein is most concentrated just within the wall (22) (20); "central bodies suggesting crushed nuclei were prominent in some varieties, obscure or absent in others" (20). "Each cluster of starch granules and, apparently each individual granule was encased in a protein film; however, the individual films were difficult to demonstrate in protein-poor areas of some varieties" (20).

Chemical analysis of rice milling fractions has added further support to the histochemical evidence for the uneven distribution of proteins in rice. First data were certainly limited to the outermost layers removed during commercial milling. A fourth of total proteins of the kernel are localised in the bran, germ and polish, which amount about 10% of the kernel weight.

Bran -reasonably free of germ- contains near the 20 per cent of the proteins of the kernel. Hulling bran contains less protein than bran. Borasio (37) reported the range 8.52-12.50% (10.1% average)(*) for the former and 13.50-16.85% (14.80% average) for the latter. Similar values have been reported by others (41). Studied fractions were obtained in a rice milling diagram in which the germ is removed by mechanical means. Protein content (total N) of bran fractions removed in successive whitening cones (14) (10) varies with degree of milling. The first fraction contains less N than the following ones; further fractions are progressively richer in N (the fourth one reached 17.6% proteins (10)).

Protein content (total N) of rice polish is similar to that of bran (64). Although some values higher for polish than for bran have been reported (65), in general, slightly lower values for the former predominate (54) (37) (66) (67) (68) (119) (134). Differences are undoubtedly influenced by the deepness and thickness of the removed fractions and especially by the presence of germ -entire and/or ground- which is more abundant in rice bran than in rice polish. The higher protein content of rice germ as compared with bran, polish and milled rice is well known (65) (37) (10) (66) (41) (86). Maymone et al (41) studied the average chemical composition of 16 samples of rice bran -from which the germ was removed at the mill- and rice polish coming from an equal number of controlled processings and found the following values for protein content ($N \times 5.95$): bran 13.9% (range: 12.4-15.8%) and polish 13.8% (range: 11.7-16.0%).

Evidence for the protein concentration gradient in bran layers has also been obtained from chemical data for milled rice. Milling and polishing of rice causes a loss of 6-30% in protein content (3) (69) (17) (2) (70) (90) (66) (72). The differences in the milling degree are the main cause of such a wide range. The protein content of the final milled rice shows less variation with milling degree than the removed bran fractions (16) (15). Adda and Rivoire (16) reported the following protein contents of rice for samples from successive whitening cones: 8.13% (for cargo rice), 8.44%, 7.97%, 7.73% and 7.76%.

Primo et al (73), in a systematic study of protein (total N) distribution in rice, investigated the protein content of layers beneath the aleurone one. They fractionated the brown rice kernel in several layers -roughly concentric- of which the first two or three ones constituted the bran and the rest were practically endosperm -milled rice. Data for the first fractions confirmed the protein distribution pattern known in bran and those for the milled rice kernel showed that: a) heterogeneity of protein distribution is not limited to bran layers, but it also occurs in endosperm, and b) a concentration gradient for proteins exists in the endosperm, the outermost region of which contains various times the concentration of protein found in the central region. The occurrence of such a protein rich outermost layer in milled rice was confirmed in further investigations carried out in this (74) (75) and other laboratories (76).

4.2.2. Protein fractions. Table II gathers in the results from several authors for protein solubility fractions content of brown and milled rice (88) (69) (89) (90) (91). It can be seen that both proteins differ in their composition, those of milled rice containing less albumins and more glutelins than those of brown rice, or which is the same, than those of bran. There

(*) $N \times 5.95$

TABLE II.- Proportion of protein solubility fractions of brown and milled rices (a)

Reference	Albumins ^(b)		Globulins ^(b)	
	Brown rice	Milled rice	Brown rice	Milled rice
Kik, 1941 (88)	-	-	-	-
Lozsa, 1955 (69)	2.28-4.33	0.60-3.36	6.09-9.24	7.25-9.99
Padmoyo et al., 1961 (89)	1.9	0.9 -1.0	11.2	1.4 -2.8
Lindner et al., 1961 (90)	2.98-18.66	0 -2.57	0- 12.23	4.65-19.87
IRRI, 1964 (91)	4.0 -11.6	2.9 - 9.9	7.9 -13.4	6.6 - 11.

Reference	Prolamins ^(b)		Glutelins ^(b)	
	Brown rice	Milled rice	Brown rice	Milled rice
Kik, 1941 (88)	3.66	5.75	44.22	40.85
Lozsa, 1955 (69)	7.38-10.01	5.33-9.43	78.50-82.60	79.89-84.61
Padmoyo et al., 1961, (89)	1.6	5.5. -8.4	85	89-91
Lindner et al., 1961 (90)	2.94-20.55	0.37-10.33	55.01-88.11	61.79-89.66
IRRI, 1964 (91)	1.6 - 4.8	1.9 - 4.2	74.3 -83.3	76.3 -87.0

(a) Data taken from (5).

(b) % of total proteins.

is not a clear pattern for the globulin and the prolamin fractions.

Proteins from bran and polish also differ in their protein solubility fractions. Although they contain similar proportions of albumins and prolamins, those from bran have more globulins and less glutelins than those from polish (91).

4.2.3.- Free amino acids. The following free amino acids have been detected in brown and milled rice: alanina, arginine, aspartic acid, cystine, glutamic acid, glycine,

histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, asparagine, and γ -aminobutyric acid (77) (78) (79) (80) (82) (83) (81) (84) (86). Glutamine was detected in brown rice (82) but not in milled rice.

In brown rice total free amino acids content range from 2.28 (adapted from (82)) to 7.30 (adapted from (86)) mg amino N/100 g rice. Predominant in amount are: alanine (10-18%), glutamic acid (9-16%), and aspartic acid (5-8%) (80)(82)(84)(86), and eventually arginine (82), glycine (82), histidine (82), leucine (82), glutamine (82), asparagine (82), serine (86), and γ -aminobutyric acid (86). Milled rice contains from 0.77 (adapted from (82)) to 6.41 (adapted from (78)) mg amino N/100 g rice. The main constituent is glutamic acid (10-51%) (78) (79) (82) (83) (85) (86). Alanine (79) (82) (83) (85) (86), leucine (78)(82), threonine (78), serine (78) (79) (83) (85) (86), aspartic acid (79) (83) (85), histidine (82), valine (82) (83), arginine (82) (83), phenylalanine (82), proline (83), cystine (83), and tyrosine (85) have also been eventually found in high proportion. In general, brown rice contains more free amino acids than milled rice. The results of Tamura and Kenmochi (86) are: brown rice, 7.30 mg amino N/100 g rice and milled rice 1.40 mg amino N/100 g rice. In both samples the main constituents were alanine, glutamic acid, and serine (amounting 50% of total amino N in brown rice, and 80% in milled rice). The major difference was that the glutamic acid content of milled rice was about four times higher than that of brown rice.

Bran and germ are rich in free amino acids. Total free amino acids in bran amount to 22 (adapted from (86)) to 300 (adapted from (78)) mg amino N/100 g flour. Bran contains practically the same individual amino acids as the milled kernel. Like in milled rice, free amino acids in bran contain high proportion of glutamic acid (7-31%) (78) (79) (81) (86); alanine (11-16%) and serine (5-15%) are other major constituents (78) (79) (81) (86) which may also be abundant in milled rice. Total free amino acids in germ amount to 24.3 (adapted from (87)) to 106.0 (adapted from (86)) mg amino N/100 g germ. The results of Tamura and Kenmochi (86) show that rice embryo contains about four times more amino N than bran, and this about twenty times more than milled rice. Major free amino acids in germ are: alanine (14%), aspartic acid (12%) proline (29.5%), and serine (12%) (86). Unlike in brown rice, milled rice and bran, aspartic acid in germ is found in relatively low proportion.

4.3. Enzymes

Systematic research on distribution of enzymes in rice is scarce. The compilation and critical study of existing data is beset with difficulties derived from non standardised methods for determining enzymatic activities and, some times, from the lack of suitable translation of Japanese papers. However, there is evidence for a heterogeneous distribution of enzymes in rice. It appears that this group of constituents shows a more acute tendency to be concentrated in determined zones than any major component in rice.

Data revealing the heterogeneous distribution of the liquefying and saccharifying components of the amylase system in the rice kernel have long been known (94) (93) (28). The data given in Table III, reported by Borasio (94), show clearly the germ and outer covers of the kernel as the sites of higher amylolytic activity. It is not elucidated however

TABLE III. Amylolytic activity of rice milling products^(*)

Products	Activity (mg maltose/10 g product)
Brown rice	39
Milled rice	15
Hulls	4
Bran	320
Polishings	250
Germ	310

(*) According to Borasio (94).

if the high activity in polishings is due to a high proportion of bran or to actual high activity in outer layers of the endosperm. According to Sreenivasan (28) the liquefying amylase (alpha-amylase) appears to be more localised in the peripheral layers and the germ than the saccharifying fraction (beta-amylase). Some other works on amylolytic activities in rough rice (64) (95) (96) (28), unpolished rice (97) (98) (99) and milled rice (100) (101) have been published, but it is difficult to make appropriate use of their data concerning amylases distribution.

Redox enzymes have also been found concentrated in localised zones. Roberts (34) reported that "in addition to the embryo, there are two layers of potentially high activity of redox enzymes, the tegmen and the aleurone layer. Positive reactions to the Nadi's reagent and to the 2,3,5-triphenyltetrazolium chloride tests of these layers indicated the presence of cytochrome oxidase and dehydrogenase activities. The dehydrogenases are found in relatively great abundance in the germ (102) (103) (104) provided germinative capacity of the seed is maintained, as shown by the tetrazolium test. A high rate of decomposition of hydrogen peroxide also shown in tegmen and aleurone, suggested the occurrence of high peroxidase or catalase activity (34).

Borasio (22), in 1929, found that catalase activity is localised in the aleuronic layer and in the germ whereas the endosperm is very poor in such enzyme. The cited author reported values showing that catalase activity ratio for brown rice: brown rice free of germ: milled rice is about 40:16:1. The more recent work of Chikasuye (105) (106) (107) on the nature and distribution of catalase in rice seeds is in agreement with these findings.

In general, outer coverings of the seed and/or especially the germ are good sources for enzymes in rice: ascorbic acid oxidase (108) (109), glutamic acid decarboxylase (64), peroxidase (110), lipase (94) (111) (112), invertase (111) (112) (113), phosphomono-esterase (114), phosphodiesterase (114) phosphatase (115) (116), phytase (115) (116), esterase

(117), dehydrogenase (102) (103) (104), cytochrome oxidase (103) (104), polyphenol oxidase (103) (104) and flavine oxidase (103) (104). However, available information on localisation of these enzymes in different zones of the kernel is fragmentary. More complete knowledge on enzyme distribution is needed for a better understanding of rice and rice products stability.

4.4. Lipids

The existing information on the distribution of lipids within the rice kernel is by far more extensive than for any other of the main components of rice. The high fat content of some rice byproducts, and the dependance of their storage life (like that of undermilled rice) upon the stability of fat have promoted much research in this direction. However, in spite of it, the existing knowledge is not only far from being complete but much less than the indispensable. The greatest part of data concern to total lipids in the kernel fraction removed during commercial milling.

4.4.1. Total fat. Histochemical evidence for the heterogeneous distribution of lipids within the kernel has been presented by various authors (13) (22) (6) (20). According to Santos(13), the pericarp and tegmen possess no fat or oil. In a more recent work (20) a material of a fatty nature, assumed to be probably suberin, has been detected in tegmen. Aleurone cells are rich in fatty material; a sheat of fat-staining material encloses the aleurone grains(20). The germ also contains fat in high proportion. Small droplets of oil occur in parenchyma cells of the plumule and the primary root, together the corresponding epithelial cells, epithelium of the scutellum, epithelial cells, inner cells of the cotyledon and epiblast(13). The endosperm is poor in fat; Borasio (22) detected by histochemical tests the existence of fat in outer layers of endosperm but he was unable to find fat in the inner portion.

Chemical analysis of rice and rice milling by-products have shown large variations in fat concentration with location in the kernel. Fat losses during milling amount to 50% to 95% (50) (17) (16) (63) (22) (44) (92) (120). According to Mori and Watanabe (71) changes during polishing in the contents of starch combined fat are negligible as compared with those of crude fat -extracted by the Schoch's procedure. The fat content of germ ranges about 10 to 25%. Than of bran is much more variable (about 5 to 30%). The latter values being influenced by the deepness and thickness of the layer. The non specified presence or absence of germ in bran is another frequent cause of variation. Bran fractions successively removed during milling vary in fat content (4) (10) (14) (124) (125). Data show that with the exception of the outermost fraction(s) which is somewhat poorer in fat than the underlying ones, fat content of bran decreases with milling degree. Fat content of rice polish, which ranges from 6 to 18% (67) (68) (37) (54) (121) (41) (51) (65), is generally lower than that of bran (67) (37) (41) (68) (54) (121), although some exceptions have been reported (51) (65) probably due to the layers considered.

The decreasing fat content of milled rice with milling degree (4) (21) (25) (92) (118) (120) (124) (125) is in agreement with above commented data for bran. It is of interest to note that the amount of fat extracted from the whole milled rice was found

to be in linear relationship to the amount of bran removed up to about 6% of the original brown rice (21) (118). Primo et al (124) have studied the variation of fat content (and other characteristics) of milled rice with the percentage of bran removed during milling. Although the pattern of variation is common for all rices investigated, absolute values of fat content for a given milling degree vary with variety and lot of rice.

Study of the distribution of fat in milled rice (4) (124) showed that heterogeneity is not limited to bran layers. Outer layers of the milled kernel are richer in fat content than inner ones. In general, there is a decreasing concentration gradient towards the center; however, one layer was found showing a lower fat content than both the next outer and inner ones. In a more recent work (24) such abnormality has also been found in American rices.

4.4.2. Lipid fractions. The scarce information available on lipid fractions of rice does not permit to know their distribution within the rice kernel.

4.4.3. Fatty acid composition of total fat. The following fatty acids have been found in brown and milled rice, rice bran, rice polish and germ: oleic, linoleic, palmitic (these three in predominant amount), myristic, stearic and linolenic acids (5) (11) (12) (31) (32) (33) (49) (53) (30) (122). In addition, lauric acid has been detected in rice bran and polished rice (33), palmitoleic acid in rice bran and polished rice (33), arachidic acid in polished rice (33), rice bran (33) (30), and embryo (12) lignoceric acid in rice bran (30) (126) (127) (128) (129), behenic acid in bran (130) and embryo (12) and an unsaturated acid $-C_{24}H_{48}O_2$ or $C_{26}H_{52}O_2-$ in bran (32).

Information on distribution within the kernel is rather scarce. The data reported by Ueno and Ueda (32) indicate that both bran and cargo rice lipids contain similar proportions of palmitic, oleic and linoleic acids, however myristic acid was only found in bran whereas stearic acid was only in cargo rice. However, the latter has been reported to be present in cargo rice by other authors (11) (33) (55). Data by Akiya and Nakayama (49) for milled rice and bran lipids showed differences in liquid fatty acids: oleic acid was more abundant in bran, whereas linoleic acid was in the endosperm. Bran lipids have been found to contain more polyunsaturated fatty acids -linoleic and linolenic acids- than milled rice lipids, whereas the latter contained greater proportions of myristic, palmitic, palmitoleic and stearic acids (33). Wu and Williams (31) did not find any fundamental difference in the fatty acid composition of total lipids from bran and polish.

4.4.4. Fatty acid composition of lipid fractions. The work of Yasumatsu and Moritaka (11) compares the fatty acid composition of phospholipids, free fatty acids and neutral fats of milled (polished) and brown rice. There is no or little difference in phospholipid and neutral fat fractions; however, the free fatty acid fraction of hulled rice contained more oleic acid and less linoleic acid than that of polished rice.

4.4.5. Chemical characteristics of rice lipids. Comparison of data for the iodine values of lipids from brown rice, milled rice, rice bran and rice polish (33) (45) (51) (123) show that, in general, the decreasing order of I.V. is: rice polish, rice bran, milled rice. Values range about 117.8 for rice polish, 82 to 109 for rice bran, and 48.5 to 73.1 for milled rice.

Saponification value is higher for lipids from milled rice than from brown rice (45) and lower for rice bran than for rice polish (15); oil from inner layers of bran showed higher saponification values than oil from outer bran layers (123).

Data reported by various authors for the acid value of lipids of different parts of the kernel or milling fractions are contradictory. Acidity is extremely influenced by the previous history of the sample, and this makes difficult to compare data from different origins. Among others, there are works in which no difference has been found in acid value among husked, undermilled and well milled rice (92) or between bran from two successive whitening cones (52). In contrast with these, some other report significant differences between rice milling fractions (131) (132). Similar comments can be made on peroxide value.

Although some other constituents of lipid nature have been reported to occur in rice (5) (12), information is so fragmentary that their distribution within the kernel can not be deduced.

PLAN OF WORK

There were several areas of research within the field of chemical composition which might yield helpful information in furthering basic studies both on aging of milled rice and rice quality. The distribution of chemical constituents was considered one of the areas of major interest. In previous investigations carried out at this laboratory (x) on the influence of the protein material in rice properties and cooking behavior of rice, the knowledge of the protein distribution within the kernel was extremely useful. The high percentage of proteins found in the outer layer of milled rice led to envisage for this constituent a more important role in rice properties than previously thought (73) (74) (75). Moreover, the knowledge of protein distribution lead to the development of a procedure for the obtention of rice flours of high nutritive value (100) (186) (187) (188) (189) (190) which promises to be of a great practical interest.

On the other hand, data for protein and other constituents examined in the Literature Review indicated that chemical composition varies throughout the endosperm.

(x) "Basic studies on the constituents of rice that influence quality and development of objective methods for measuring market quality of raw and precooked rice", project no. UR-E25-AMS-1(a), grant no. FG-Sp-107-60.

Therefore, a comprehensive knowledge of such distribution was needed in order to know the actual reactivity and stability of milled rice, particularly of its outermost layer. The outer layer should reasonably be more liable to modification both during aging and technological processes than any other region of the kernel.

The scarcity of information on distribution of chemical constituents within the milled rice kernel (existing data were practically limited to total nitrogen and fats) made a systematic study of main rice components convenient.

Starch is the major constituent of rice and this justifies in to be included in present study. The behavior of starch in hot water is responsible in a great extent for the properties of the cooked rice grain. Proportion, composition and properties of rice starch differs from variety to variety and some of such differences have been related to rice behavior on cooking and processing. However differences in starch distribution have not been taken into consideration.

Sugars are present in milled rice in minor amounts, as indicated by average chemical composition data. However, as shown in the Literature Review, there is some evidence that sugars in outer layer may reach relatively high levels, which might be worthy of study. The knowledge of sugars distribution is of particular interest in connection with the study of storage changes. Qualitative and quantitative changes of sugars have been associated with deterioration of raw (43) (135) (136) (138) (139) and treated rices (43) (137) (140). In investigating these changes, the average sugar content of the entire kernel has been the basis. However, if it is substantiated that outer and inner parts of the kernel differ greatly in sugar content, a revision of the problem discriminating the changes in both parts might afford interesting knowledge. Changes in the more easily damaged outer layer should reasonably be different.

Interest for proteins has already been mentioned above. On the other hand, data in the bibliography for brown and milled rice samples suggest that not only protein content but also protein composition varies with the deepness of the layer. Therefore, the existing knowledge on total N distribution deserves to be extended, discriminating protein N, non-protein N, free amino N, protein solubility fractions, etc., which must differ in their properties and influences on rice behavior. The quantity as well as the quality of the nitrogenous material should be taken into account.

The enzymatic systems occurring in milled rice, their variations and their effects on the characteristics and properties of the kernel is one of the more interesting features in elucidating the mechanism of the rice aging reaction. The dependence of both activity of these biological catalysts and storage life of rice upon environmental conditions suggests a close association. Characteristics of gross chemical constituents of rice change during storage, affecting the properties of the kernel. The role played by enzymes in these changes is not known. Knowledge on enzyme distribution in milled rice is meager. Data for other grains suggest that enzymes in rice should also be heterogeneously distributed; even regions practically devoided of enzymatic activities are envisaged. It means that data on enzyme levels determined for the entire kernel are of little value. The occurrence of enzymes in localised parts of the kernel -which might be of actual practical significance- should be investigated.

Finally, the lipids are of major interest regarding storage changes. Their unstability affects substantially the storage life of rice. Fat deterioration brings about development of off-flavors, increased acidity, and other changes not well known yet that affect rice quality and decrease the overall acceptability of the cereal.

The stability of lipids is closely associated to their chemical nature; the polyunsaturated components of fats oxidize many times faster than do the monoethenoid and saturated acids. On the other hand, location of lipids in the rice kernel must play an operative role. The rapid deterioration of undermilled rice derivates from particular favorable conditions: high concentration of lipids, high levels of enzymatic activities, direct contact with atmospheric oxygen, microflora concentration, etc. Hence, and because of their possible participation in cooking behavior, it is of basic interest to obtain a more complete knowledge of the chemical nature and distribution within the milled kernel of rice lipids.

Present work is limited to the study of the distribution within the rice kernel of the following constituents (×):

1. Starch. 1.1. Amylose. 1.2. Amylopectin. 1.3. Total, reducing and non-reducing sugars. 1.4. Individual sugars.

2. Nitrogen compounds. 2.1. Total, protein and non-protein nitrogen. 2.2. Protein solubility fractions. 2.3. Sulfhydryl and disulfide groups. 2.4. Free amino nitrogen.

3. Enzymes. 3.1. Alpha-amylase. 3.2. Beta-amylase. 3.3. Proteolytic activity. 3.4. Cysteine desulhydrase. 3.5. Cystine reductase.

4. Lipids. 4.1. Total lipids. 4.2. Lipid fractions: neutral fats, free fatty acids and phospholipids. 4.3. Fatty acid composition of each three fractions. 4.4. Chemical characteristics of lipids: acid, saponification, ester, iodine, peroxide and TBA values.

The investigations approached two different features: a) distribution of constituents in layers of different deepness, and b) composition of outermost layer as compared with that of nucleus and entire kernel of milled rice. The purpose of part a) studies was mapping the constituents in the rice kernel, thus affording a general knowledge of actual chemical composition of rice. For that, thin layers were successively removed from dehulled rice in a special abrasion mill and the composition of each was studied with detail. The objective of part b) studies was to obtain a thorough knowledge of the chemical composition of the outermost layer of milled rice, in order to achieve a better understanding of its reactivity and properties. This layer plays an operative role -different from that of the rest of the kernel- in rice behavior. It is the frontier with environment. Its knowledge was considered fundamental in furthering studies on rice behavior during cooking, technological processes and storage. Furthermore, a comparative study of the outermost layer, the

(×) Histochemical and additional chemical work is reported in Part III.

nucleus and the entire kernel was also considered pertinent. It should help to discriminate the influence on rice properties of outer from inner portions of the kernel. On the other hand, it should allow to relate the new information based on the outer layer's data with the existing knowledge on the characteristics and properties of rice based on the average chemical composition of the entire kernel.

EXPERIMENTAL

1. DISTRIBUTION OF CONSTITUENTS WITHIN THE RICE KERNEL.

MATERIALS

The following rice varieties and samples were used: Balilla, 1963 crop, and Bailla x Sollana, 1964 crop, in carbohydrate studies; Balilla x Sollana, 1964 crop, in lipid studies; Balilla and Bomba, 1964 crop, in studies on sulfhydryl and disulfide groups; Balilla x Sollana, 1966 crop, in studies on enzymes. All samples were grown at the "Estación Arrocería", in Sueca, Valencia.

Milling of samples was carried out at an experimental mill ("Torrejon", Valencia, Spain) previous dehulling in a laboratory rubber-roll shelling machine ("Imad", Valencia, Spain).

Removal of successive layers of hulled and milled rice samples was carried out at a laboratory abrasion mill similar to that developed by Hogan et al (76); processing was carried out under nitrogen atmosphere. When necessary, hulled rice was carefully degermed by hand.

METHODS

1. CARBOHYDRATES

1.1. Starch, amylose and amylopectin.

a) Deffating of rice flour fractions.- Obtained samples were defatted according to the procedure followed by Wagenknecht (141). Chloroform-ether extracts were removed and the defatted flour was dried in vacuum at room temperature.

b) Determination of starch.- The official method of the A.O.A.C. (154) was used.

c) Determination of amylose and amylopectin.- The method of Taki (142) was followed.

2. NITROGEN COMPOUNDS

2.1. Sulfhydryl and disulfide contents.- They were determined according to the method of Axford et al (143).

2.2. Protein content.- It was determined by the method of U.S.P. (144). Results are given on a dry basis. Nitrogen was transformed into proteins multiplying by 5.95.

3. ENZYMES

3.1. Determination of alpha-amylase activity.- The colorimetric method of Sven Hagberg (145) with slight modifications according to the AACC (146) was used.

3.2. Determination of beta-amylase activity.- It was determined by extraction of the former according to Tipples and Tkachuk (147), beta-amylolysis of a standard substrate following the procedure of Manners et al (148) and final determination of resulting sugars by 3-5 dinitrosalicylic acid (149).

3.3. Determination of proteolytic activity.- It was determined by the method of the AOAC (150), using hemoglobin as standard substrate.

4. LIPIDS

4.1. Extraction of lipids.- Flour samples were defatted, immediately after separation, with chloroform: methanol 2:1, according to the procedure of Wagenknecht (141). Extractable lipids are given as total lipids.

4.2. Fractionation of lipids.- The lipids were separated into free fatty acid, neutral fat, and phospholipid fractions. The latter fraction was isolated by removing neutral fats and free fatty acids with acetone according to the procedure of Wagenknecht (141). Neutral fat fraction was extracted from its mixture with the free fatty acid fraction using ethyl ether-petroleum ether (1:1) according to Mattick and Lee (151). Saponification was carried out according to the method of Lee and Mattick (152). Methyl esters of fatty acids of each lipid fraction were prepared using diazomethane as described by Giral (153).

4.3. Gas chromatography.- For the GLC analysis of the purified methyl ester preparations a Perkin-Elmer 820 was used, the column was a B.D.S. (8%) Chromosorb W, HMDS (80-100 mesh), 6 feet, 1/8" Ø; condition were: column 200°C, injector 270°C and detector 230°C.

II. CHEMICAL COMPOSITION OF THE OUTER LAYER AS COMPARED WITH THOSE OF THE NUCLEUS AND THE ENTIRE KERNEL.

MATERIALS

Balilla x Sollana rice variety, 1965 and 1966 crops, grown in Sueca, Valencia, was used. Milling of samples and removal of successive layers from the kernel were carried out as described previously.

METHODS

1. CARBOHYDRATES

1.1. Starch and its constituents.- Starch was determined by the method of the AOAC (154). Amylose was determined by amperometric titration with iodine (155). Results for amylose were calculated using the iodine binding capacity of crystalline rice amyloses given by Phillips and Williams (156). Samples were dissolved according to the method of Williams et al. (157).

1.2. Sugars: a) Total, reducing, and non-reducing.- The official method of the AOAC (150) was used.

b) Individual Sugars.- 1) Extraction. The following procedure was used: extraction of rice flour with boiling 70% aqueous ethanol (158), removal of alcoholic solvent under vacuum, clarification of the extract passing in through Hyflo Super Cell and deionization by passing through ion exchange columns (159) and concentration of extract under vacuum. 2) Gas-liquid chromatography. Trimethylsilyl derivatives of sugars were prepared with use of hexamethyl-disilazane as described by Brobst and Lot (160). For GLC analysis a Perkin-Elmer F.7 was used, with flame ionization detector (FID). Column used was a 5% silicone (SE-52) on Celite 545 (60-100 mesh); 2 m long and 2.7 mm I.D. Conditions were: columns temperature varied from 125°C to a maximum of 270°C, by using a temperature program at 2.5°C per min.; nitrogen carrier gas flow rate, 75 c.c. per min. 3) Paper chromatography. Concentrated sugar solutions were chromatographed on Whatman n° 1 paper using the solvent system pyridine: ethyl acetate: water (4:12:3). Spots were developed by spraying either with resorcinol in l-butanol or silver nitrate in ammoniacal solution (159).

2. NITROGEN COMPOUNDS

2.1. Total N, non protein N and protein N.- Total N was determined by the U.S.P. Kjeldahl's method (144). Non-protein N was analysed by the method described by McIntyre (161); N content of extracts was determined by Kjeldahl's micro-method (154). Protein N was calculated by mathematical difference between total N and non protein N.

2.2. Protein solubility fractions.- The procedure of Sturgis et al (162), modified by Cagampang et al (163) was used. Nitrogen in the extracts was determined by Kjeldahl's micromethod (154).

2.3. SH and SS groups.- They were determined according to the method of Axford et al. (143).

2.4. Total free amino N.- The modified method of Sorensen was used (146).

3. ENZYMES

3.1. Determination of alpha-amylase activity.- See Section 1.3.1. above.

3.2. Determination of beta-amylase activity.- See Section 1.3.2. above.

3.3. Determination of proteolytic activity.- See Section 1.3.3. above.

3.4. Cystine reductase.- The procedure described by Romano and Nickerson (168) was used.

3.5. Cysteine desulphydrase.- It has been determined by the method of Smythe (169).

4. LIPIDS

4.1. Total lipids, lipid fractions and fatty acid composition of lipids were determined by the procedures described above (See Section 1.4).

Lipid characteristics.- They were determined as follows: acid value by the AOAC method (150); saponification value by the IUPAC method (164); iodine value by the Wijs method (164); peroxide value by the micromethod of Vioque and Vioque (165); TBA test, by Tarladgis et al method (166); total carbonyl contents by the procedure of Henick et al (167).

RESULTS AND DISCUSSION

I. DISTRIBUTION OF CONSTITUENTS WITHIN THE RICE KERNEL.

1. CARBOHYDRATES

1.1. Distribution of starch, amylose and amylopectin

Starch content of layers successively removed from the dehulled rice kernel are shown in Table IV. As it can be seen, there is an increasing concentration gradient from outer regions towards the center of the kernel. The low starch content of outermost

TABLE IV.- Distribution of starch within rice kernel (Balilla variety)

Layer	% of layer	% starch in layer
I (a)	6.37	29.43
II	4.11	41.24
III	7.79	61.38
IV	5.91	67.32
V	11.31	73.20
VI (b)	64.51	92.28

(a) outer layer

(b) nucleus.

layer (I) reflects the presence in this flour fraction of most of the coverings of the kernel and the germ, which are practically devoid of starch (13) (39) (40) (26). However, it is not only due to their diluent effect but to the scarcity of this constituent in subaleurone layers, that such a low value is found, as shown by data for deeper layers obtained after removal of bran. Data obtained previously for proteins (total N) (73) and lipids (4) support these results. Present data also are in agreement with those previously published by Normand et al (24) for starch content of 12 successively removed fractions of commercially milled long-grain rice. The American researchers noted that there is an inverse relationship between starch and protein-lipids content of the fractions. McCall et al (170) reported previously, based on average chemical composition data of the entire kernel for different varieties and environmental conditions, that there is a highly significant negative correlation between starch and nitrogen contents.

The determination of amylose and amylopectin contents of different layers of the kernel demands special considerations. Conventional procedures involve iodine titration and calculations are based on the iodine combining capacity of well purified amylose and amylopectin samples used as reference substances. Such procedures would be extremely time-consuming and present various inconveniences derived from amylose and amylopectin purification. On the other hand, it has been reported (156) that amylose of entire -milled- rices bind 18.9% of iodine. However, some fluctuations -assumed to be due to varietal effect- have been found (171). Moreover, still is unknown whether starch fractions of different layers of the kernel have or not the same iodine combining capacity. Differences in size and other characteristics between starch granules from outer and inner regions of the kernel have been reported (20) (6).

Due to all this, a method not requiring purified starch fractions as reference substances would be desirable. The method developed by Taki (142) appeared appropriate

for this purpose. It consists in fractionating the starch by paper chromatography and colorimetric determination of the isolated fractions by the anthrone sulfuric acid method. The oxidant and strongly acidic medium during fractionation might involve partial modification, but rigorous control of processing conditions should permit reasonably good results provides the comparative purpose of them. However, in spite of the great number of data accumulated, it has not been possible to withdraw definite conclusions. Results are not sufficiently reproducible and, on the other hand, the recovery of starch constituents throughout the fractionation process is too low, ranging from 85% to 95%. Being in mind these considerations, the great quantity of experimental data accumulated allow us only to withdraw some tentative conclusions—meanwhile a more critical examination of the method used may be carried out. Available data show that: a) amylose and amylopectin concentration increase from outer to inner layers of the kernel in a similar way as it happens with starch^(*), and b) the proportion of both fractions in starch is similar at every layer. These results are in contrast with more recent data (24) obtained by colorimetry of the starch-iodine complex^(**), which indicate that the amylose content of rice starch progressively increase from outer to inner regions although exceptions were found in some layers.

Distribution of sugars has also been studied. However, unlike other subjects dealt with in present chapter, sugars were not investigated systematically in successively removed layers. The investigation consisted in the comparative study of the outer layer and the nucleus of rice samples with different milling degrees. Therefore, the information obtained will be presented in a later chapter (See Section II.1.2.).

2. NITROGEN COMPOUNDS

2.1. Distribution of sulfhydryl and disulfide groups

In previous studies—which are described later on in Part III some evidence was found showing that sulfhydryl and disulfide groups might play a role in the behavior of rice during cooking. Remarkable differences in SH and SS contents were found among nine rice varieties investigated, and the existence of a highly significant positive correlation between SS groups and rice quality was shown; the SS content was generally higher in varieties of better cooking quality (174). On the other hand, previous studies carried out at this laboratory^(***) (172) (74) (173) showed that of the proteins in the milled kernel those

(*) Amylose content of each layer was also determined by amperometric titration (155). Values obtained in this way showed the same pattern of distribution for amylose than Taki's data.

(**) The method used was that of Mc Cready and Hassid (175) as modified by Williams et al (157), using 18.9% iodine combining capacity of pure crystalline amylose for calculation.

(***) The investigation was supported by a grant from the U.S. Department of Agriculture, Agricultural Research Service and Agricultural Marketing Service.

located in the outer layer are the most influencing the rice cooking behavior. However, no information was available on SH and SS content of proteins in peripheral layers.

Therefore, it was considered of interest to determine the SH and SS distribution within the rice kernel before continuing the study of the relationship between these functional groups and rice properties. If SH and/or SS groups occur in much higher concentrations in the outermost layer than in inner regions, it appears reasonable to search their possible influence on rice in such a layer. Moreover, the knowledge of SH and SS distribution in the rice kernel also has a basic interest, as there is not information on this subject.

3.1.a. SH and SS contents of layers of different deepness. Results obtained for SH and SS contents of successively removed layers of dehulled rice -Bomba variety- are given in Table V. Concentration of these functional groups varies with the deepness of the layer. The distribution found is shown in Fig. 2. With the exception of the outermost layer -containing most of the true bran-, the concentration of SH groups as well as that of SS groups decreases from outer regions towards the center of the kernel.

The pattern of distribution of SH and SS groups in rice parallels therefore that previously found for proteins (73). Cystine content of successively removed fractions of conventionally milled rice has been determined by Normand et al. (24) in a recent work. Their results show that, with the exception of the first fraction, there is a progressive decrease in the cystine content of the layers as the center of the kernel is approached. Although not directly comparable, cystine data distribution in milled rice adds further support to the distribution previously found for SH and SS groups. The technique of Schram et al. (176) followed by the cited authors for determining amino acids, converts cysteine and cystine into cysteic acid, the value of which is expressed as cystine. Cystine content data for bran, polishings and milled rice reported by various authors (66) (65) (121) are in agreement with the above results.

Differences among layers are remarkable. As it can be seen in Fig. 2 -and it should be noted with interest- the highest concentration of SH and SS groups occurs in layer II, which constitutes the outermost layer of the milled rice grain. This situation might be of importance. The outer layer determines in a great part the quality of rice. Being the SH and SS concentration in the outer layer high -about three times greater than the average concentration in the entire kernel- it is more than likely that these functional groups located in outer layer influence cohesiveness of the cooked kernel surface. It is known that in wheat flour the SH and SS groups of the polypeptide chains are involved in mechanisms influencing rheological characteristics of baking dough.

The level of SH and SS in the outer layer may also be of interest in connection with chemical and physicochemical changes taking place in rice during storage. Liability to oxi-reduction reaction of these functional groups is high and their location in the outermost layer makes changes even more feasible. Remarkable changes in the characteristics of rice produced by storage (or moderate technological processes), which may take place without appreciable variations in the gross chemical composition, might find a reasonable explanation

TABLE V. - Distribution of sulfhydryl and disulfide groups in the rice grain.

LAYER		DISULFIDE GROUPS		SULFHYDRYL GROUPS		PROTEIN (g)		
No. of the grain (a)	Proportion of the grain (b)	Protein	Rice	Protein	Rice	%		
		-S-S- (c)	-S-S- (d)	-SH (e)	-SH (f)			
BOMBA VARIETY								
I	8.95	40.89	7.29	17.29	18.45	3.29	16.57	17.83
II	4.74	46.08	8.81	11.07	20.08	3.84	10.24	19.12
III	6.23	36.30	6.12	10.10	20.17	3.40	11.92	16.86
IV	80.08	31.66	2.90	61.54	14.85	1.36	61.27	9.16
Grain	100.00	33.46	3.77	100.00	15.75	1.77	100.00	10.89
BALILLA VARIETY								
I	6.96	40.98	6.59	16.14	17.28	2.78	15.71	16.08
II	5.41	37.13	5.28	10.05	16.95	2.41	10.59	14.22
III	9.17	30.59	3.27	10.56	19.74	2.11	15.71	10.69
IV	78.46	31.46	2.29	63.25	13.34	0.91	57.99	7.28
Grain	100.00	32.34	2.84	100.00	14.40	1.22	100.00	8.58

(a) No. I is the outermost layer

(b) g layer/100 g husked rice, dry basis

(c) μ eq -SS-/g of protein(d) μ eq -SS-/g of layer(e) μ eq -SH/g of protein(f) μ eq -SH/g of layer

(g) g protein/100 g rice, dry basis

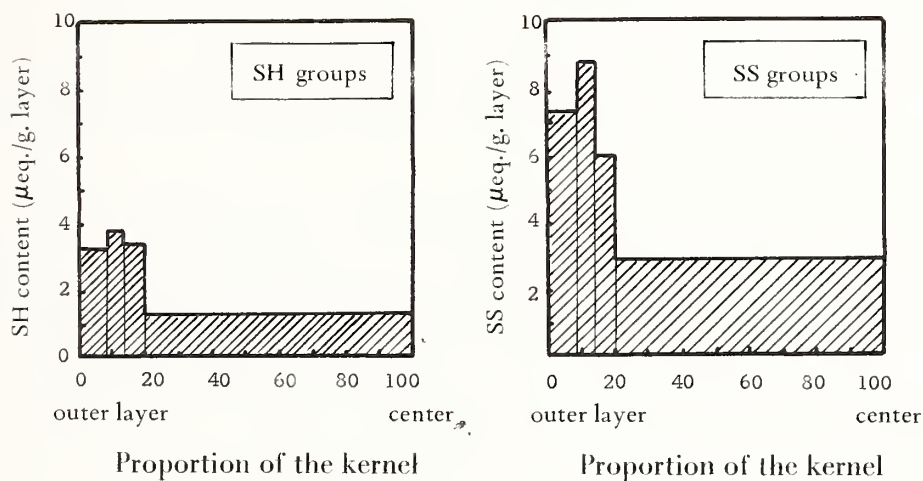


Fig. 2.- Diagrams showing the SH and SS distribution within the rice kernel

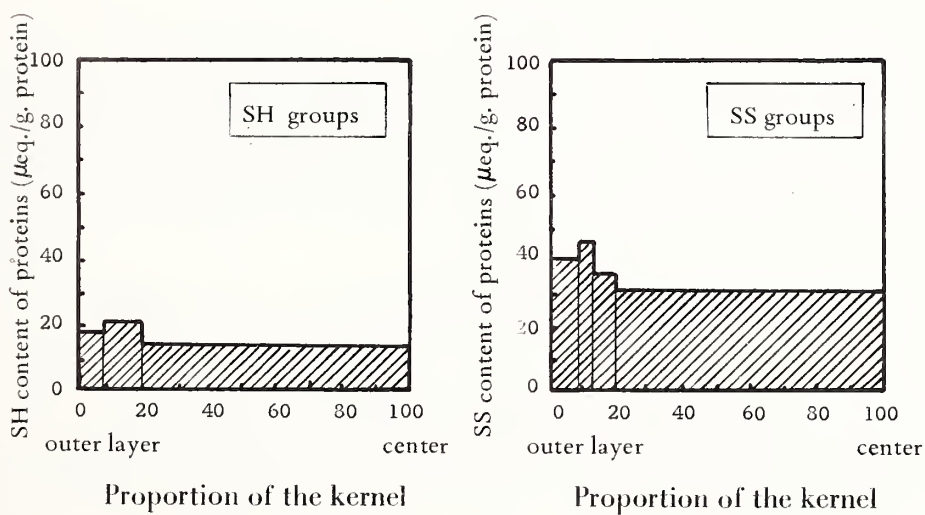


Fig. 3.- Diagrams showing the variation of SH and SS contents of proteins with layer deepness within the kernel.

through mechanisms involving these groups. These aspects of the problem are dealt with in Part III:

2.1.b. Sulphydryl and disulfide contents of proteins from different layers of the kernel. From results for SH, SS and total N, proximate SH and SS contents of proteins in every layer were calculated. Results are included in Table V and plotted in Fig. 3. As it can be seen, the chemical nature of rice proteins varies with their location in the kernel. Proteins in the peripheral layer contain less sulphydryl groups and, especially, less disulfide groups than the near underlaying layer; then, after reaching a maximum, proteins become progressively poorer in SH and SS contents as they approach the center of the grain. It is of interest to note that layer II -which approximately corresponds, at least in part, with the outermost layer of commercially milled rice- presents both the highest protein concentration and the proteins of highest SH and SS contents.

2.1.c. Varietal differences in SH and SS contents. Distribution of sulphydryl and disulfide groups was determined in several rice varieties. Table V report data for two of them, Bomba and Balilla. The pattern of distribution is the same in both rices (Fig. 4). Balilla rice does not show the peak concentration as Bomba rice does. Most probably the layer with highest SH and SS is not so deep as in the case of the Bomba rice and it is included in layer I. In wheat as well as in corn, the SH peak occurs in aleurone layer (177).

Comparison of the SH and SS distributions in Bomba and Balilla varieties is of interest because both rices differ remarkably in their cooking quality. The former is a first quality rice; its kernels remain entire, undisrupted, firm and non cohesive after cooking. The latter is a poor quality rice (x); its kernels do not swell too much, become somewhat disrupted showing a wide suture line, and have a tendency to stick each other.

As it can be seen in Fig. 4 the SH and SS concentrations in every layer are higher in Bomba rice than in Balilla rice. Differences are particularly remarkable at peak levels. In layer II, comprised between 6% and 12% of milling -which includes the outermost layer of commercially milled rice- the SH contents are: Bomba, $3.84 \mu \text{ eq/g}$ rice and Balilla, $2.41 \mu \text{ eq/g}$ rice; the SS contents also are quite different: 8.81 and $5.28 \mu \text{ eq/g}$ rice respectively.

Differences in the chemical composition of the proteins from both rices are also apparent (Fig. 5); however they are smaller.

The information presented suggested a possible role to be played by the SH and/or the SS groups of the outer layer in the behavior of rice during cooking and processing, which was subsequently investigated (See Part III).

3. ENZYMES

Modern investigation on enzymatic activities in rice, especially on their distribution within the kernel, is scarce as shown in the Literature Review. Amylolytic enzymes occur in rice which are able to breakdown starch molecules and bring about

(x) Eventually, Balilla samples from some isolated harvests and particular regions of Spain have been studied in this Laboratory, they being of an unusual good quality.

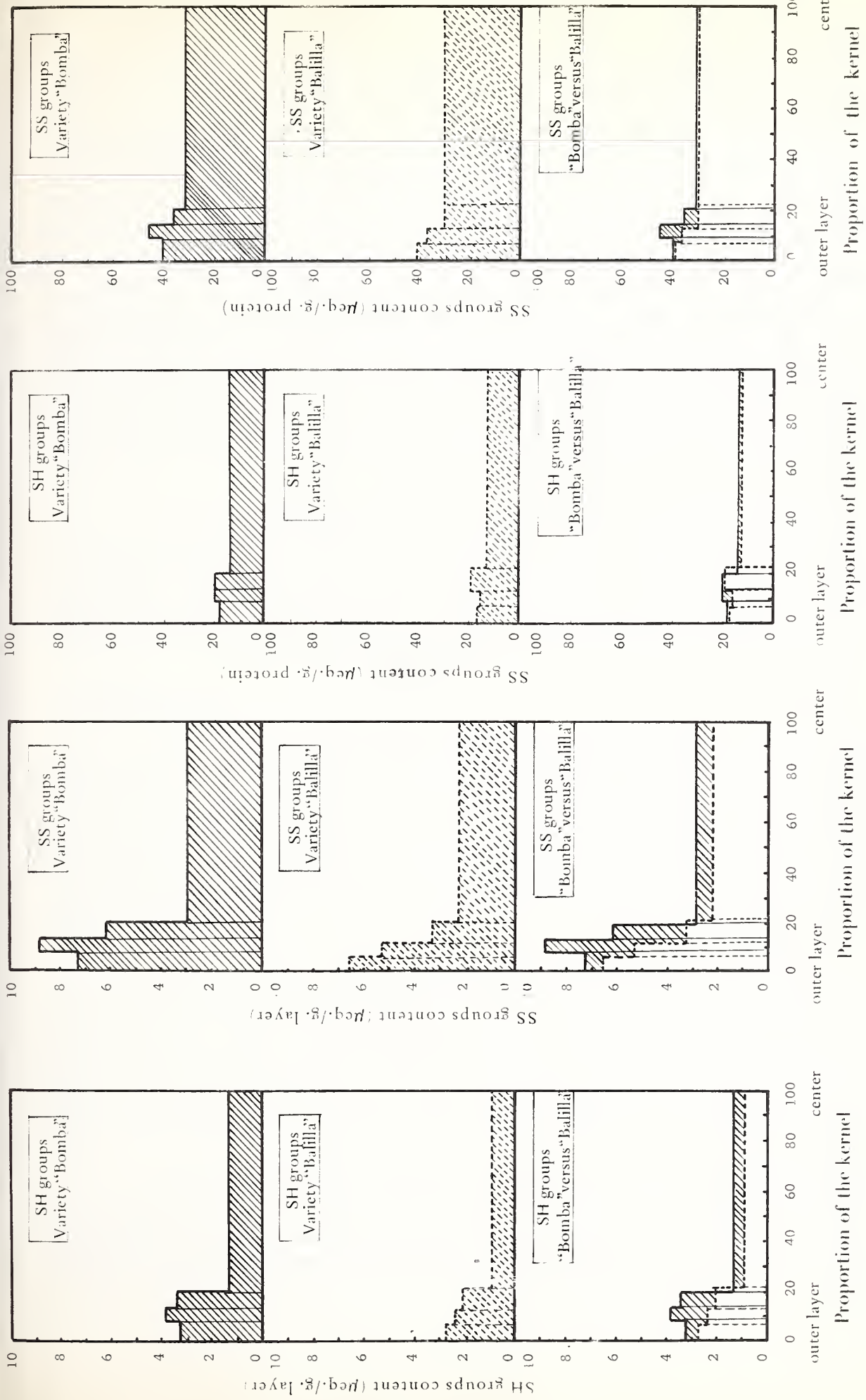


Fig. 4.- Diagrams showing varietal differences in the SH and SS distribution within the rice kernel

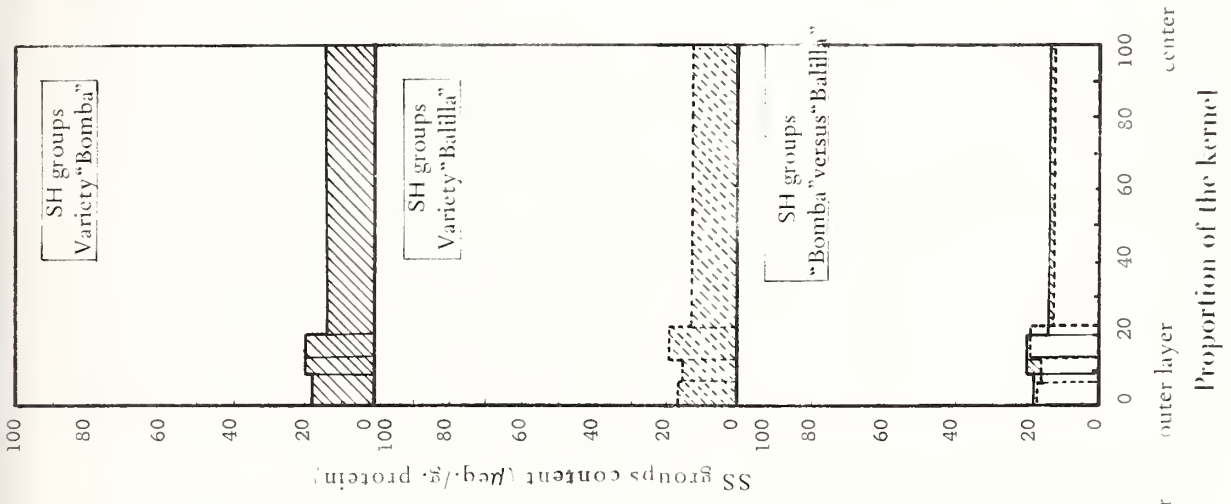


Fig. 5.- Diagrams showing varietal differences in SH and SS contents of proteins in layers of different deepness

remarkable changes in this major component. There is some evidence that higher enzymatic levels are localised in outermost layers of the kernels, the possible changes produced being therefore of a greater significance in rice properties. Lipids are liable to oxidative rancidity and hydrolysis. Enzymatic catalysis is involved in both types of deterioration. In parallel studies on compositional changes during aging of rice -which are described later on in Part II- it has been shown that the chemical characteristics of proteins undergo significant changes. At present, it is unknown if enzymatic systems, with specific action on certain proteins or on their constituents are involved in these and other related changes. For instance, it was found in previous studies -cited above- that SH and SS groups occur in high concentration levels in the outer layer of rice. Later on, it was found that storage brings about significant changes in them (see Part II). The fact that SS groups of cystine can be reduced by the catalytic action of cystine reductase to SH groups, and the latter can be transformed by cysteine desulphydrase to SH_2 moved us to investigate the occurrence of these enzymes in rice.

When investigating enzymes and their distribution within the kernel, especial attention was paid to those affecting the major constituents of rice. Out of them, enzymes from which information was scarce or envisaged to be more closely related to cooking quality, were selected. These were: alpha-amylase, beta-amylase, proteolytic activity, cystine reductase and cysteine desulphydrase. The latter two were only determined in outer layer, nucleus and the entire kernel and results will be presented in the corresponding section.

3.1. Alpha-amylase

Brown rice, hand-degermed, was processed in a laboratory abrasion mill (see Experimental) and four peripheral successive layers removed. Alpha-amylase activity in the four fractions, the residual nucleus and the original entire kernel were determined. Results are given in Table VI and plotted in Fig. 6. The data show a decreasing concentration gradient from the outer layer towards the central position of the kernel. Alpha-amylase activity is mainly concentrated in the outermost layer. This, which comprises the 5.89% of the hand-degermed dehulled kernel weight, contains almost 45 per cent of total alpha-amylase activity of the kernel.

In wheat and in other cereals such as rye and barley (178) (179) the pericarp and aleurone layers contain little or no amylase. If this would be true for rice, the actual alpha-amylase activity of the outer region of the endosperm (subaleurone layer) should be very much higher than that found for layer I (0.94 SKB units). True pericarp and aleurone layer amount to about 5 per cent of the kernel weight (8); therefore, a major part of layer I must be constituted by them. However there is some indication (28) (94) (181) (182) of that alpha-amylase is not absent in outer covers of the kernel.

As it can easily be seen in Fig. 6 alpha-amylase activity decreases sharply with layer deepness. Its level in layer II is about one half of that found in layer I. The same is true with layer III as compared with layer II. Combined I and II layers represent somewhat more than the portion removed in commercial milling. More than 60 per cent of total alpha-amylase activity of the entire kernel -free of germ- is therefore removed at the mill.

TABLE VI. Distribution of alpha-amylase, beta-amylase and proteolytic activities within the rice kernel^(a)

Layers		Alpha-amylase activity		Beta-amylase activity		Proteolytic activity	
(b)	Proportion of the kernel (%)	in layer (c)	Proportion of total activity in the kernel (%)	in layer (d)	Proportion of total activity in the kernel (%)	in layer (e)	Proportion of total activity in the kernel (%)
I	5.89	0.94	43.91	147.34	20.09	17.37	61.56
II	4.64	0.54	19.80	193.53	20.79	4.94	13.79
III	3.13	0.25	6.20	98.05	7.12	1.04	1.96
IV	8.60	0.08	5.45	87.03	17.33	0.77	3.98
V	77.74	0.04	24.65	19.26	34.67	0.40	18.71
Entire kernel (a)(f)	100.00	0.13	100.00	43.18	100.00	1.66	100.00

(a) Hand-degermed brown rice

(d) mg. maltose/g. rice, d.b.

(b) No. I: outermost layer

(e) Hemoglobin units/g. rice, d.b.

(c) S.K.B. units/g. rice, d.b.

(f) Calculated values

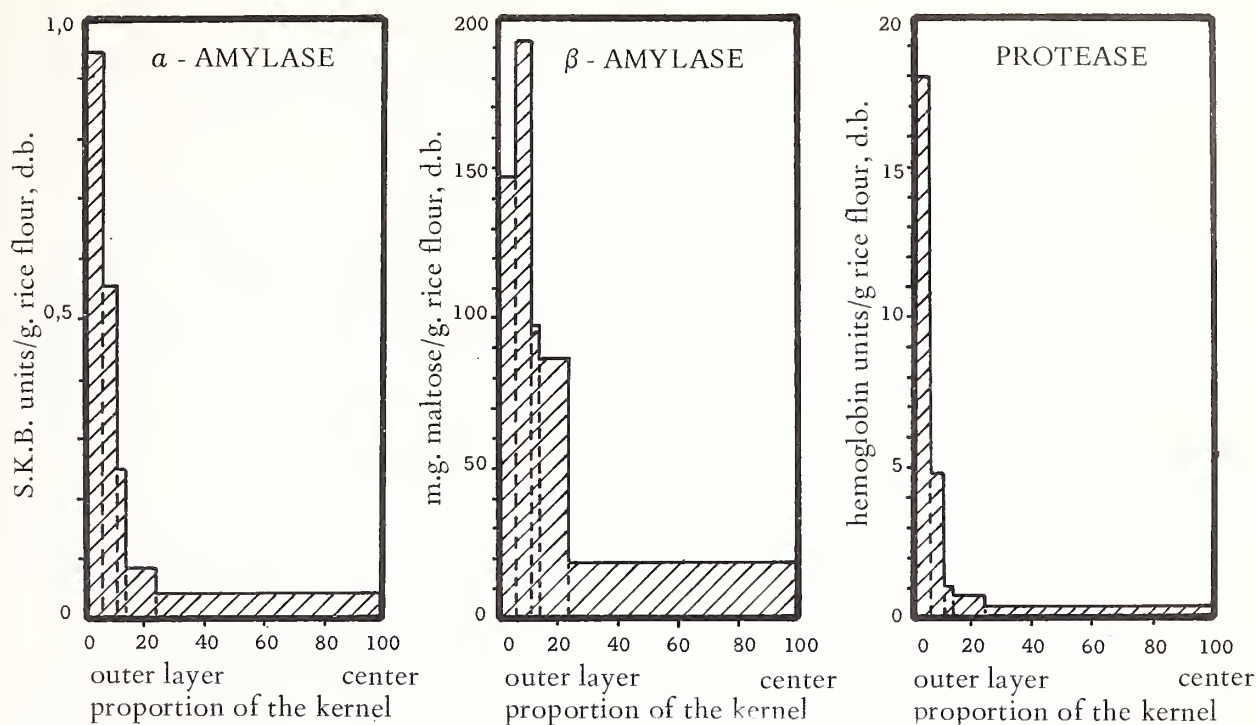


Fig. 6.- Distribution of α -amylase, β -amylase and proteolytic activities within the rice kernel

Data for layers III, IV and V are in fair agreement with the distribution found by Houston et al (100) in a sample of commercial milled Calrose rice.

Alpha-amylase activity of the germ was determined in a sample of the same rice variety as used for distribution studies (Balilla \times Sollana), although of different previous history. The level found in germ (6.87 SKB units) was much higher than any of those in kernel layers. Giri and Sreenivasan (181), Yamagishi (182) and Borasio (94) reported previously that liquefying and saccharifying components of the amylase system are mostly localised in the germ (and peripheral layers).

3.2. Beta-amylase

Beta-amylase showed a distribution similar to that of alpha-amylase. (Table VI, Fig. 6). A minor difference was that beta-amylase level at the surface layer was lower than in layer II. Houston et al., (100) did not find a peak in beta-amylase activity as they worked on milled rice. Mc Masters et al., (180) reported values for wheat showing that the inner portion of the starchy endosperm had a higher activity than the outer one.

As compared with the alpha-amylase activity, that of beta-amylase decreases more slowly towards the center of the kernel. The nucleus (layer V) accounts for 34.6 per cent of total beta-amylase activity whereas only for 24.6 per cent of total

alpha-amylase activity. Conversely, removal of the two first outer layers represents a loss of 40 per cent of the total beta-amylase whereas more than 60 per cent of the total alpha-amylase. This is in good agreement with previous authors (181) (182) who reported that alpha-amylase in rice is concentrated in more outer layers than beta-amylase.

3.3. Proteolytic activity

The enzyme appears to have a similar distribution pattern to that of alpha- and beta-amylases, that is, high levels in the outer layers which decrease sharply towards the center of the kernel (Table VI, Fig. 6). The outermost layer, representing the 5.89 per cent of the kernel weight accounts for 61 per cent of the total proteolytic activity. The level in layer III is notoriously low (1.04 hemoglobin units).

These results are similar to those published for wheat and other cereals (178) (179) (183). According to Engel (178) there is "a fairly large amount of proteinase in the aleuronic cells and no measurable quantity in the endosperm".

Proteolytic activity in the germ, (determined in another rice sample), was very high: 31.77 hemoglobin units. Pett (183) found larger amount of proteinase in the axis and scutellum than in the endosperm of wheat. However Engel (178) reported that "in the germ, there was so little proteinase that it could not be determined in the different parts".

4. LIPIDS

This work, which is a continuation of a previous one on the distribution of total lipids in rice (4)*, consists in a systematic study of the composition of lipids localised in layers of different deepness within the rice kernel. As it has been seen in the Literature Review, there is a lack of such knowledge, it in spite of its basic interest in connection with rice behavior during cooking, processing, and, especially, storage of milled rice. As cited in the Plan of Work, present section comprises the following studies: 4.1. Distribution of free fatty acids, neutral fats and phospholipids. 4.2. Fatty acid composition of a) free fatty acids, b) neutral fats, and c) phospholipids of successively removed layers of the kernel.

4.1. Distribution of free fatty acids, neutral fats and phospholipids

The contents of free fatty acids, neutral fats and phospholipids decrease from outer to inner regions of the grain, just as it happens with total lipids (Table VII).

In the entire kernel, and in each layer, the concentration of neutral fats is far higher than that of the other two fractions. Thus, for instance, the neutral fat contents of layer I (the outermost one) is 15.30%, whereas those of free fatty acids and phospholipids are 0.78 and 1.24%, respectively.

* This work was part of a research project supported by a grant from the U.S. Department of Agriculture, Agricultural Research Service and Agricultural Marketing Service.

TABLE VII. Distribution of free fatty acids, neutral fats and phospholipids in the rice grain (Balilla x Sollana variety)

LAYER		TOTAL LIPIDS (e)		LIPID FRACTIONS				
No. (a)	Proportion of the grain (b)	% in layer	Proportion of the lipids in the grain	% in layer (c)			Proportion of the total lipids in each layer (d)	
				Free fatty acids	Neutral fats	Phospho- lipids	Free fatty acids	Neutral fats
I	4.89	17.32	49.81	0.78	15.30	1.24	4.52	88.31
II	2.74	11.56	18.62	0.58	9.73	1.24	5.03	88.22
III	11.63	2.95	20.18	0.31	2.46	0.18	10.57	83.24
IV	80.74	0.24	11.39	0.06	0.15	0.03	27.03	60.96
Grain (b)	100.00	1.70	100.00	0.14	1.43	0.13	7.88	84.01

(a) No. I is the outermost layer

(d) g of lipid fraction/100 g of total lipids in the layer

(b) Grain of husked rice, without germ

(e) Chloroform: methanol (2:1) extractable lipids, according to

(c) g of lipids/100 g of layer, dry basis

Wagenknecht, (141).

Although the distribution pattern of the three lipid fractions is similar, neutral fats and phospholipids show a relatively sharper decrease than free fatty acids. In outer layers, the concentration of phospholipids is higher than that of free fatty acids, whereas in inner layers it is lower.

The proportion of free fatty acids, neutral fats and phospholipids in total lipids changes with location in the kernel. Lipids in inner layers contained less neutral fats and more free fatty acids than those in outer layers. Thus, for instance, the free fatty acids which in the outermost layer represent 4.52% of the total lipids, in the center of the grain (layer IV) represent 27.0%.

It does not seem likeable that these results may be effected by a hydrolytic degradation of the lipids during the preparative process. If any possibility of alteration does really exist, it must be very small and it looks as if it should not have any substantial influence. Flours were extracted immediately after each fraction was obtained at the mill. The separating methods used were mild.

On the other hand, the proportion of free fatty acids (calculated from data in Table VIII) for after removing layers I and II, agrees well with the data found by Yasumatsu and Moritaka (11) for milled rice of Asiatic varieties. Similar results have also been found by Lee et al. (184) for American and Asiatic varieties.

Phospholipids do not show a regular way of distribution. Neither do they show it in their fatty acid composition, as it will be seen later on. Whether their existence in the rice grain really happens in this way or not, this is a question that deserves further confirmation based upon a more detailed investigation. Separative techniques (185) may provide a not too accurate evaluation of the phospholipids, due to the presence of substances that do not contain any phosphorus but may be acetone insoluble -like some cerides-, or of acetone soluble phospholipids -like some sphingolipids-.

4.2. Fatty acid composition of lipids in layers of different deepness

The fatty acids found in a larger amount in all fractions and in all layers, were: palmitic, oleic and linoleic acids. Minor components were: capric, myristic, palmitoleic, stearic and linolenic acids (Table VIII). All these acids, with the exception of capric acid, have been previously reported by other workers as components of the lipids of rice and/or of their by-products (husked rice, milled rice, bran and/or polishings) in American and Asiatic varieties (11) (31) (32) (33) (184). Fatty acids corresponding to 12:0 (33), 20:0 (33) (184), 20:3, 24:0 and 16:2 (184) have not been detected in the studied rice. These acids, however, have been found in American and Asiatic varieties in a small proportion, generally as traces.

4.2.a. Free fatty acids. (Table VIII). In this fraction, acids corresponding to 16:0, 18:1 and 18:2 are predominant in amount. The former, the palmitic acid, shows very little change from one layer to another; it is somewhat more abundant in the outermost layer, where it generally represents a fifth of total free fatty acids. The unsaturated acids 18:1 and 18:2 show quite an

TABLE VIII. Fatty acid composition of free fatty acids, neutral fats, and phospholipids in successive layers of the rice grain

L A Y E R		L I P I D F R A C T I O N (d)		F A T T Y A C I D C O M P O S I T I O N (c)						
No. (a)	Proportion of the grain (b)	% in layer	Proportion of the total lipids in each layer (e)	F R E E F A T T Y A C I D S						
				10:0	14:0	16:0	16:1	18:0	18:1	18:2
I	4.89	0.78	4.52	-	1.3	24.6	2.2.	2.1	36.9	32.9
II	2.74	0.58	5.03	2.5	1.4	21.7	2.1	1.6	29.5	41.2
III	11.63	0.31	10.57	1.4	0.8	21.5	Tr	1.3	24.5	49.7
IV	80.74	0.06	27.03	-	-	21.5	Tr	Tr	16.5	62.0
Grain(b)	100.00	0.14	7.88	0.65	0.71	22.51	0.48	1.10	25.77	48.58
N E U T R A L F A T S										
I	4.89	15.30	88.31	-	0.3	16.1	0.4	0.9	37.3	44.0
II	2.74	9.73	88.22	-	0.3	16.6	-	0.8	38.2	43.1
III	11.63	2.46	83.24	-	0.4	16.8	Tr	0.6	40.5	40.5
IV	80.74	0.15	60.96	-	1.0	21.6	1.1	0.9	24.8	49.6
P H O S P H O L I P I D S										
I	4.89	1.24	7.16	-	0.3	31.6	2.0	1.4	39.7	25.0
II	2.74	1.24	10.73	-	-	15.8	Tr	1.7	46.3	36.2
III	11.63	0.18	6.18	Tr	3.4	20.7	10.6	6.6.	44.7	12.0
IV	80.74	0.03	11.99	-	-	26.5	5.5	5.7	30.0	32.3

(a) No. I is the outermost layer

(d) Taken from Table VII

(b) Grain of husked rice, without germ, dry basis

(e) g of lipid fraction/100 g of the total lipids in layer

(c) The number to the left of the colon (:) denotes the length of the carbon chain, and the number to the right of the colon represents the number of double bonds in the fatty acid.

interesting distribution. Oleic acid concentration progressively decreases from outer layers to the center of the grain; in layer I it represents 36.9% of the free fatty acids, while in layer IV it only represents 16.5%. Linoleic acid, on the contrary, is more abundant in the center portion of the kernel (32.9% in layer I and 62.0% in layer IV). In the outer layer, therefore, the proportion of oleic acid is similar to that of linoleic acid, but it is nearly four times lower in the center.

These results are in good agreement with the data published by Yasumatsu and Moritaka (11). The said authors found out that the proportion of palmitic acid in the free fatty acid fraction of husked rice and milled rice was quite similar. They also found that the said fraction -free fatty acids- contained more oleic acid and less linoleic acids in husked rice than in milled rice.

The concentration of 14:0, 16:1 and 18:0 acids decreases towards the center of the grain. (One of them the myristic acid, even disappears; its presence could not be detected in layer IV). They are therefore distributed like oleic acid.

Capric acid is found, almost exclusively, in the free fatty acid fraction. It has not been detected as component of neutral fats.

From data obtained for every layer, the fatty acid composition of the free fatty acid fraction of husked rice (free of germ) have been calculated. Results have been included in Table VIII. Palmitic acid content of the grain is similar to that of oleic acid (23.09% and 25.77%, respectively), the existing differences among layers being compensated. The total content of linoleic acid is twice that of palmitic or oleic acids.

It should also be mentioned that the proportion of unsaturated acids is practically constant in all layers studied and, consequently, similar to the total proportion in the entire kernel, it being approximately 75-80% of free fatty acids.

4.2.b. Neutral fats. (Table VIII). The 16:0, 18:1, and 18:2 acids are, like in other fractions of lipids, the main components of neutral fats. They represent 96-98% of the fatty acids in this fraction. The unsaturated acids 18:1 and 18:2 represent 75-80%.

The 16:0, 18:1 and 18:2 fatty acid composition of neutral fats is practically the same in the first three layers of the grain. However, the proportion of oleic acid decreases from 40.5% in layer III to 24.8% in layer IV. That of linoleic acid increases. The proportion of palmitic acid is higher in layer IV than in all other layers.

It is interesting to point out that the changes in the fatty acid composition of neutral fats when passing from layer III to layer IV, coincide with a variation in the neutral fats content of total lipids. While in the three first layers neutral fats represent, respectively, 88.31, 88.22 and 83.24% of lipids in layer IV they only represent 60.96%.

In this fraction, no capric acid has been detected. The fatty acids 14:0, 16:1, 18:1 and 18:3 have been found in small proportion, about 1% or less; no important differences

have been observed from one layer to another.

4.2.c. Phospholipids. (Table VIII). The predominant characteristic of fatty acid distribution of phospholipids is irregularity. The acids corresponding to 16:0, 18:1 and 18:2 are, like in other fat fractions, the most abundant components; their proportion changes from one layer to another and does not keep any relationship with the deepness of the layer.

It has also been noticed that 16:1 and 18:0 acids appear in proportions substantially higher than in the other two lipid fractions; palmitoleic acid, for instance, represents 10.6% of the fatty acids of phospholipids in layer III, while in the same layer, but in the free fatty acid and neutral fat fractions, it is found in the proportion of traces.

It should be noted too, the absence of the fatty acids 10:0, 14:0 and 18:0 in phospholipids of some layers.

The heterogeneous distribution of lipids, and the variation of their chemical composition with the location in the kernel, shows that the average composition data for the entire kernel provide an inaccurate knowledge which does not reflect the real condition of the lipids in the kernel.

II. CHEMICAL COMPOSITION OF OUTER LAYER AS COMPARED WITH THOSE OF NUCLEUS AND ENTIRE KERNEL OF MILLED RICE.

The information accumulated on the distribution of constituents within the rice kernel showed that heterogeneousness in composition does not disappear with commercial milling. Composition of the rice grain varies from the pericarp to the center of the kernel. In general, changes are sharp at the outer layers and mild in inner ones.

This knowledge allows to differentiate in the milled rice kernel two parts -outer layer and nucleus-, the chemical properties of which are expected to be different, and, therefore, are worthy to be studied and discriminated.

With this purpose, the chemical composition of these kernel fractions was investigated. The information obtained is reported in present section. At the same time, the chemical composition of the milled entire kernel was studied with comparative purposes.

1. CARBOHYDRATES

1.1. Starch and its constituents

Table IX reports starch and amylose contents of outer layer (10 per cent of the entire kernel), nucleus and entire kernel of undermilled (7.7% of milling) and well milled (12.0%) rices. As it was expected the outer layer in both rice samples contains less starchy material than the nucleus. It is of interest to point out that differences between outer and

TABLE IX. Chemical composition of milled rice: comparison of outer layer, nucleus and entire kernel. I. Starch and its constituents.

a) Starch and amylose contents			
	Entire kernel	Outer ^(a) layer	Nucleus
<hr/>			
Undermilled rice ^(b) :			
Starch (%)	83.29	67.40	85.00
Amylose (%)	8.82	6.11	9.68
Milled rice ^(c) :			
Starch (%)	86.62	74.77	88.37
Amylose (%)	11.05	9.49	11.21
<hr/>			
b) Distribution of starch and amylose in outer layer and nucleus as % of total contents in entire kernel			
<hr/>			
	In outer layer (a)	In nucleus	
<hr/>			
Undermilled rice ^(b) :			
Starch	8.1	91.9	
Amylose	6.5	93.5	
Milled rice ^(c) :			
Starch	8.6	91.4	
Amylose	8.6	91.4	

(a) 10% of the weight of the entire kernel

(b) 7.7% of milling

(c) 12.0% of milling

inner regions in starch are greater than those generally found among common rice varieties; they amount to 17.6%, in undermilled rice and 13.6%, in well milled rice. Differences in amylose content are smaller. It suggests a higher proportion of amylose in starch of inner layers as compared to that of the outer layer. This is in agreement with the data reported by Normand et al (24). In this connection, it should be mentioned that in both cases amylose determination was based on the 18.9% iodine combining capacity of crystalline amylose (156) (see previous Section I, 1, 1.).

Data given in Table IX indicate different chemical reactivity and properties in outer layer and in nucleus, not only because of the lower starch content of the former but also its higher content in other constituents -fats, proteins, enzymes. On the other hand outer layer data show more differences between samples of different milling degree than entire kernel data, supplying a more actual knowledge of their composition and properties.

1.2. Sugars

Reducing, non reducing and total sugars have been determined in an outer layer representing 10% or 5% of the kernel weight, the residual nucleus and the original entire kernel of rice samples of three different milling degrees - 7.78%, 9.8% and 12.0%. In addition, individual sugars in the outer layer (5%), the nucleus, and the entire kernel of the medium milled rice sample have been investigated.

1.2.a. Reducing, non reducing and total sugars. Table X reports total (TS), reducing (RS) and non reducing sugars (NRS) contents of outer layer, nucleus and entire kernel of rice samples of different milling degree. TS content of the entire kernel is low; values range from 0.61 to 0.25%, they being between ordinary limits published for milled rice (5). TS content of outer layer and of nucleus differs from that of the entire kernel, and from each other too. In undermilled rice, the outer layer contains 4.02% TS and the nucleus 0.17% TS. Concentration in outer layer is, therefore, about 30 times higher than in nucleus and about 6 times that of the entire kernel. In samples of higher milling degree differences also are significant although smaller. TS are, therefore, not uniformly distributed in milled rice; there is a decreasing concentration gradient from outer to inner layers. This is in agreement with the lower sugar content of brown rice as related to milled rice (42). In this connection, it should be mentioned that in brown rice, the outermost layer is poorer in TS than the near underlaying one (10). It will have been noticed that sugar distribution within the rice kernel parallels that for proteins, whereas is opposite to that for starch.

The decrease in TS concentration is sharp in peripheral layers and slight in the inner region. The major part of TS is localised in a very thin outer layer. Thus, in undermilled rice, the outermost layer of only 0.1 mm thick, contains 72.7 per cent of TS of the entire kernel. In medium milled rice, the 0.03 mm thick outer layer contains almost 45 per cent of TS.

The RS/NRS ratio also varies with layer deepness. NRS decrease faster than RS. NRS are predominant in amount in outer layer; in the undermilled sample they represent 87.6% of TS. In the nucleus, RS and NRS are similar. In the entire kernel, the influence of the outer layer pattern predominates. These results are in fair agreement with published data for the average chemical composition of the entire kernel (135) (136) (138) (139).

It seems convenient to point out that sugar composition patterns may be modified during storage. Rice undergoes changes not only in TS, but also in RS and NRS, which may be non compensated.

TABLE X. Chemical composition of milled rice: comparison of outer layers nucleus and entire kernel. II. Sugars.

a) Total, reducing and non reducing sugars content			
	Entire kernel	Outer layer ^(a)	Nucleus
Undermilled rice (7.7%)			
Reducing sugars (b)	0.14	0.50	0.08
Non reducing sugars (c)	0.47	3.52	0.09
Total sugars (d)	0.61	4.02	0.17
Milled rice (12.0%)			
Reducing sugars (b)	0.08	0.37	0.07
Non reducing sugars (c)	0.17	0.86	0.06
Total sugars (d)	0.25	1.23	0.13
Medium milled rice (9.8%)			
Reducing sugars (b)	0.12	0.50	0.07
Non reducing sugars (c)	0.26	2.42	0.11
Total sugars (d)	0.38	2.92	0.18
b) Distribution of sugars in outer layer and nucleus as % of total contents in entire kernel.			
	In outer layer ^(a)	In nucleus	
Undermilled rice (7.7%)			
Reducing sugars	41.0	59.0	
Non reducing sugars	81.2	18.8	
Total sugars	63.1	36.9	
Milled rice (12.0%)			
Reducing sugars	37.0	63.0	
Non reducing sugars	61.4	38.6	
Total sugars	51.3	48.7	
Medium milled rice (9.8%)			
Reducing sugars	27.8	72.2	
Non reducing sugars	53.8	46.2	
Total sugars	46.1	53.9	

(a) 10% of the whole undermilled and milled kernel, 5% of the medium milled kernel

(b) g maltose/100 g rice, dry basis

(c) g sucrose/100 g rice, dry basis

(d) g/100 g rice, dry basis

1.2.b. Individual sugars^(*). Sucrose, glucose and fructose are the main sugars; they account for almost total sugars in rice. Other five sugars have been found in minor amounts; out of them only two, xylose and galactose, have been identified. Trace amounts of arabinose (and/or rhamnose) and maltose have also been tentatively identified. An important peak, showing the shortest retention time, has been identified as glycerol.

Identification of constituents has been based on the relative retention volume (α) related to alpha-glucose in GLC and R_f related to glucose in paper chromatography. Reference values were obtained with standard sugar mixtures chromatographed in identical conditions. The following values were obtained for sucrose: $\alpha = 1.87$ and $R_{gl} = 0.74$. GLC conditions did not allow for a good separation of sucrose from lactose ($\alpha = 1.88$), but in the paper chromatogram the spot considered as sucrose gave positive reaction when treated with resorcinol (159). Two peaks, well differentiated, were found for alpha- and beta-glucose. Retention volume for the beta-isomer was 1.12. The proportion of both constituents in TS of outer and inner regions of the kernel was similar but differed from that of equilibrium for both isomers. Fructose gave $\alpha = 0.89$ and $R_{gl} = 1.15$. Although fructose could not be differentiated from arabinose in the paper chromatogram, GLC was decisive (arabinose: $R_{gl} = 1.15$; $\alpha = 0.63$). Xylose gave $\alpha = 0.75$ and galactose $\alpha = 0.94$. Isolation of galactose was difficult by paper chromatography, due to the great quantity of sucrose present. Arabinose and rhamnose gave $\alpha = 0.63$ and $\alpha = 0.64$ respectively. This region in the GL chromatogram showed three peaks, too small for reliable identification. Finally, maltose showed two peaks ($\alpha = 2.03$ and 2.07) which seem to correspond with the alpha- and beta-isomers, having the alpha-maltose the lowest retention volume (160).

Sugars found in the investigated rice sample have been previously reported to be present in various rices from different origins. Sucrose, glucose and fructose: (42), (191) -except sucrose-, (5) (192), (43), (47), (48); arabinose: (5); galactose: (5), (43); maltose: (43), (192), (193), (47); xylose (as well as glycerol): (5).

The reported individual sugars were found in both the outer layer and the nucleus. The only differences between both kernel fractions were: concentration of sugars in flour and proportion of individual sugars in total sugars. Sucrose accounted for almost the total amount of the non reducing fraction and was most abundant in quantity and proportion in the outer than in the inner region of the kernel. Reducing sugars were mainly constituted by glucose and fructose. Proportion of these constituents varied with the deepness of layer, glucose being more important in the outer region.

It has been reported (42) (191) that glucose is the most important reducing sugar in rice, accounting for almost all this sugar fraction. In the sample investigated here, fructose was present in considerable quantity; in the outer layer, it accounted for more than 30 per cent of total reducing sugars. It is now unknown if this is due to the previous storage of the rice sample used or to a varietal character.

(*) This work was done after months of storage. As compared with the non stored sample, some variation may have taken place.

2. NITROGEN COMPOUNDS

2.1. Total N (NT), non protein N (NPN) and protein N (PN)

NPN in rice amounts to 20-40 mg N/100 g flour. Its concentration varies with the deepness of layer, the outer region containing twice as much as the nucleus (Table XI). Therefore, NPN distribution parallels that of total N. Neither its quantity nor its distribution may, therefore, change the assumption that TN reflects PN in the rice kernel, in a similar manner as it happens in wheat (196).

In the investigated sample, NPN is about 1-2 per cent of TN, which is in good agreement with previously published data for milled rice (69) (195). According to Jodidi (194), NPN in brown rice amounts to 4-5% of TN.

As a % of TN, NPN in outer layer is higher than in nucleus (1.78% and 1.36% respectively). This is in agreement with results obtained previously (Table XII) for under- and well milled rices, and data published by Lozsa (69) for husked and polished rices.

Data for PN (table XI) were as expected from the well known TN distribution. In spite of the high milling degree of the sample investigated, protein content of outer layer was very high (14.80%).

As it can be seen in Table XI, the outer layer, 0.03 mm thick, contained about the 10 per cent of the TN, NPN and PN of the whole kernel.

2.2. Protein solubility fractions

The extraction procedure of Sturgis et al. (162) has been modified recently by Cagampang et al. (163) with improved extraction efficiency. This modified procedure has been used in present work. The protein extraction yields were as follows: outer layer 77.9%, nucleus 80.7% and entire kernel 79.3%. These are in fact higher than the mean 65 per cent yield obtained in our previous studies with the Sturgis procedure (75), but are slightly lower than those reported by Cagampang et al., (163).

It will have been noticed that extraction yield in outer layer flour is somewhat less than in nucleus; the difference is perhaps too small to be taken into consideration. However attention is drawn to it here because a similar situation is inferred from the data of Cagampang et al. (163) for the contents of protein and protein fractions in brown rice milling fractions of low- and high-protein samples of three varieties. As calculated from their data, the cited authors obtained higher extraction yields for milled rice flour than for rice polish, the differences being significant. If this is due to differences in physicochemical nature of proteins or in type of flour particle is unknown.

Data given in Table XI show that the concentration of every protein fraction in outer layer is much higher than in nucleus. The highest level corresponds to glutelins. This

TABLE XI. Chemical composition of milled rice: comparison of outer layer, nucleus and entire kernel. III. Nitrogen compounds.

a) Content in nitrogen compounds.			
	Entire kernel ^(f)	Outer layer ^(a)	Nucleus
Total N (b)	1.390	2.532	1.268
Non protein N (b)	0.019	0.045	0.018
Protein N (b)	1.371	2.487	1.250
Protein fractions (c)	Albumins	1.75	0.29
	Globulins	1.12	0.60
	Prolamins	0.72	0.22
	Glutelins	7.93	5.05
	Insoluble fraction	3.28	1.48
Free amino N (d)	3.40	25.11	2.55
SH groups (e)	1.27	3.30	1.17
SS groups (e)	4.50	12.85	4.02

b) Distribution of N compounds in outer layer and nucleus as % of total content in entire kernel.

	In outer layer (a)	In nucleus
Total N	9.5	90.5
Non protein N	11.7	88.3
Protein N	9.9	90.1
Protein fractions	Albumins	75.7
	Globulins	91.1
	Prolamins	85.0
	Glutelins	92.4
	Insoluble fraction	89.6
Free amino acids	34.2	65.8
SH groups	13.0	87.0
SS groups	14.4	85.6

(a) 5% of the milled kernel

(b) g/100 g rice, dry basis

(c) N \times 5.95; g/100 g rice, dry basis

(d) mg amino N/100 g rice, dry basis

(e) μ eq/g rice, dry basis

(f) 9.8% milling degree

TABLE XII. Chemical composition of milled rice: comparison of outer layer, nucleus and entire kernel of rice samples with different milling degree. III. Nitrogen compounds.

	Undermilled rice (a)			Milled rice (f)		
	Entire kernel	Outer layer(b)	Nucleus	Entire kernel	Outer layer(b)	Nucleus
Total N (c)	1.356	2.347	1.139	1.274	1.820	1.087
Glutelin N (c)	0.420	0.772	0.385	0.421	0.806	0.387
SH groups (d)	1.39	2.19	1.12	1.33	2.30	1.05
SS groups (d)	4.22	7.45	3.45	3.54	6.51	3.20
Free amino N (e)	10.51	41.05	4.40	4.71	16.70	3.34

(a) 7.7% of milling

(b) 10% of the milled kernel

(c) g/100 g rice, dry basis

(d) μ eq/rice, dry basis

(e) mg/100 g rice, dry basis

(f) 12.0% of milling

fraction accounts for 70-80% of total extracted proteins. Attention may be drawn to the concentration of albumins in the outer layer which is six times that of in the nucleus, whereas other protein fractions are only 2-3 times higher in the outer than in the inner region of the kernel. Near 25 per cent of total albumins of the kernel is found in the outer layer.

The accumulation of albumins in the outer layer appears to be of particular interest in many respects such as nutritive value, cooking quality and storage, as suggested by the following facts: a) this fraction is the protein which contains more lysine than any other protein in rice (90) (197) (198); b) albumins are rich in cystine and sulfhydryl containing proteins (12)(197) (198), and c) many albumins may be enzymes (in wheat, amylase appears to be present in the albumin fraction (199)).

The ratio albumins: globulins: prolamins: glutelins varies with the deepness of layer. In the outermost region is 15:10:6:69 whereas in the nucleus is 5:10:4:81. These results are consistent with those of Houston et al. (200) (201) (202) for the distribution of protein solubility fractions in the milled rice kernel. Our data also are in agreement with those reported by Cagampang et al. (163) for bran, rice polish and milled rice, and by various authors (69) (89) (90) (163) for brown and milled rices of different varieties. When data from the cited various authors are compared, it is observed that there is a general agreement concerning albumins and glutelins location within the kernel, but not concerning prolamins and globulins.

2.3. Sulphydryl and disulfide groups

Data for SH and SS contents given in Table XI confirm that these groupings occur in higher levels at the outermost region. The 5% outer layer contains near the 15 per cent of all SH and SS groups in the kernel.

Values for the outer layer were higher than those found in distribution studies with two varieties (Table V). SS level for the entire kernel ($4.5\mu\text{eq SS}/100\text{ g rice}$) was also higher than those obtained in a previous study for nine varieties (174); then, the highest SS content was found in Bomba rice ($4.36\mu\text{ eq SS}/100\text{ g rice}$). On the other hand, SS index (x) of the rice sample now studied was lower than that of Bomba rice: 1702 v. 2250 respectively (see Part III). All these results could nevertheless be reasonably explained on the assumption that the rice now examined has a more flat SS distribution curve.

Proteins from outer layer had higher SH and SS contents than those from nucleus: 22 v. $15\mu\text{ eq SH/g}$ and 86 v. $54\mu\text{ eq SS/g}$ respectively. These differences are consistent with data obtained previously (Table V), although absolute values are higher.

2.4. Free amino N (FAN)

Data for outer layer and nucleus of rices with different milling degree (Tables XI and XII) show that distribution of free amino N within the rice kernel is heterogeneous. Free amino N concentration in outer layer is several times that of in nucleus, like it happens with TN, PN and NPN. The NAL concentration gradient is sharp at the outer layers; consequently, milling degree remarkably affects the average NAL content of milled rice.

Our results are in agreement with data published by various authors for brown rice, germ, bran and milled rice (86) (78) (79), according to which free amino acids occur in greater proportion in the outer layers removed during milling.

As it can be seen in Table XI, the 5% outer layer of milled rice contained near 35 per cent of total FAN in rice. The occurrence of such a high concentration in surface layers must facilitate the ready extractability and great losses during cooking.

FAN content of the entire kernel amounts about 0.25 per cent of TN. However, this proportion is not maintained throughout the kernel: it is higher in outer layer (0.98%) than in nucleus (0.20%).

(x) Surface corrected values (173).

3. ENZYMES

Data for alpha- and beta-amylase and proteolytic activities in outer layer, nucleus and entire kernel are reported in Table XIII A. As expected from the distribution previously found, the enzymatic activities were much higher in outer layer than in nucleus. An important proportion of the total enzymes in the kernel is contained in the 5% outermost layer (Table XIII A). Attention may be drawn to the high percentage of alpha-amylase in this layer. It agrees well with the more external location of this enzyme shown in distribution studies.

Data given in Tables XIII A and B show that enzymic activity per gram of protein is also higher in the outer layer than in the nucleus. The differences are remarkable, particularly as it concerns proteolytic activity.

The occurrence of cysteine desulhydrase and cystine reductase has also been investigated. Data for the former (Table XIII B) revealed that: 1st) cysteine desulhydrase occurs in both fractions and 2nd) there is a decreasing concentration gradient towards the center of the kernel, in a similar way as it happens with alpha- and beta-amylase and protease. Data found supply further evidence of the greater unstability of the outer layer as compared with that of the nucleus.

The occurrence of cysteine desulhydrase activity in rice seems of interest for several reasons: a) it suggests a possible mechanism for SH and occasionally SS losses during storage, b) it indicates a cause of flavor and color deterioration in rice. SH_2 has been found to contribute to the typical flavor of cooked rice (203). In aged raw rice, it is therefore a point to be taken into consideration. On the other hand, the evolution of SH_2 and occurrence of metals in the outer layer of the kernel, suggest a way of discoloration of rice. In this connection it seems of interest to comment that color changes during storage, as followed by the Hunter color difference meter, have repeatedly shown that color fading of milled rice involves a variation towards the red and the yellow components, the latter showing the greatest change. Cadmiun, present in rice (204), could perhaps contribute to this yellowing.

If the cysteine desulhydrase activity in rice is inherent to the kernel or is due to microorganisms (i.e. *Xantomonas*) has not been investigated.

Attempts to demonstrate the occurrence of cystine reductase in rice have not been successful. However, it is of interest to note that qualitative test for glutathione reductase activity appear to be positive.

4. LIPIDS

The following items were studied in outer layer, nucleus and entire kernel of rice samples of three different milling degrees (7.7%, 9.8% and 12.0%): a) total lipids (TL), b) lipid fractions -free fatty acids (FFA), neutral fats (NF) and phospholipids (F)-, c) fatty acid composition of lipid fractions and d) chemical characteristics of total lipids. Tables XIV to XVII report the results obtained.

TABLE XIII A. Chemical composition of milled rice: comparison of outer layer, nucleus and entire kernel. IV. Enzymes.

a) Levels of alpha-amylase, beta-amylase and proteolytic activities			
	Entire kernel	Outer layer(a)	Nucleus
alpha-amylase (b)	0.115	1.03	0.07
beta-amylase (c) (e)	44.89	223.81	31.26
protease (d)	0.89	6.03	0.63

b) Distribution of enzymes in outer layer and nucleus as % of total content of entire kernel.		
	Proportion of total	
	In outer layer(a)	In nucleus
alpha-amylase	44.3	55.7
beta-amylase	27.4	72.6
protease	33.5	66.5

(a) 5% of kernel weight	(d) Hemoglobin units/g rice, d.b.
(b) SKB units/g rice, dry basis	(e) Sample A-1
(c) mg maltose/g rice, dry basis	

TABLE XIII B. Cysteine desulphydrase activity in milled rice

Cysteine desulphydrase activity (U/g)	Bomba	Balilla x Sollana
Entire kernel	-	19
Outer layer	71 ^(a)	65 ^(b)
Nucleus	20	-

(a) 5.2% of the kernel weight

(b) 5.5.% id.

TABLE XIV. Chemical composition of milled rice: comparison of outer layer, nucleus and entire kernel. V.A: Lipids.

a) Lipids and lipid fractions contents			
	Entire kernel(d)	Outer layer(a)	Nucleus(a)
Total lipids (b) (c)	0.66	4.44	0.45
Free fatty acids (b)	0.21	1.34	0.15
Neutral fats (b)	0.38	2.53	0.26
Phospholipids (b)	0.07	0.57	0.04

b) Distribution of lipids in outer layer and nucleus as % of total content of entire kernel.

	Proportion of total	
	In outer layer(a)	In nucleus
Total lipids	34.2	65.8
Free fatty acids	32.1	67.9
Neutral fats	33.8	66.2
Phospholipids	42.8	57.2

(a) 5% of the kernel weight

(c) Chloroform:methanol (2:1) extractable lipids

(b) g/100 g rice, dry basis

(d) 9.8% milling degree

4.1. Total lipids. Lipids content of outer layer is remarkably high. It depends greatly on milling degree. The 10% outer layer of 7.7% milled rice contains 5.28% fat whereas that of the 12.0 milled rice only 2.24% (Table XVI). Because of the sharp decreasing concentration gradient within outer layers, the thinnest the outermost layer considered the highest the lipid contents is, and the more actual idea of the fat barrier may be achieved.

The lipids content of the 5% outer layer of milled rice (Table XIV) suggest for such component a role quite different from that of the entire kernel does. This is of interest in connection with rice cooking behavior and stability of rice during storage.

4.2. Lipid fractions. NF is the most abundant fraction in both outer layer and nucleus (Table XIV). The reverse happens with phospholipids. The 5% outer layer, only 0.03 mm thick, contains more than a third of the total FFA, NF and F of the entire kernel. Phospholipids appear to be more concentrated towards the outer layer than the other fractions.

TABLE XV. Composition of lipids of milled rice: comparison of lipids in outer layer, nucleus and entire kernel.

		Entire kernel(g)	Outer layer(a)	Nucleus(b)
Free fatty acids (c)		31.31	30.03	33.33
Neutral fats (c)		58.63	57.16	57.78
Phospholipids (c)		10.06	12.81	8.89
Fatty acid composition of "free fatty acid fraction" (d) (e)	12:0	Tr	Tr	Tr
	14:0	0.42	0.21	0.52
	16:0	18.72	18.32	19.55
	16:1	Tr	Tr	Tr
	18:0	1.11	0.90	1.19
	18:1	25.96	37.47	19.71
	18:2	51.72	41.06	56.90
	18:3	2.07	2.04	2.13
	20:0	Tr	Tr	Tr
	12:0	Tr	Tr	Tr
Fatty acid composition of "neutral fats fraction" (f) (e)	14:0	0.53	0.53	0.53
	16:0	18.17	16.56	19.04
	16:1	Tr	Tr	Tr
	18:0	1.28	1.79	1.15
	18:1	33.47	39.76	28.59
	18:2	45.10	40.09	48.99
	18:3	1.45	1.27	1.70
	20:0	Tr	Tr	Tr

(a) 5% of the milled kernel

(b) By calculation

(c) g/100 g lipids

(d) g fatty acid/100 g free fatty acids

(e) The number to the left of the colon denotes the length of the carbon chain, and the number to the right of the colon represents the number of double bonds in the fatty acid.

(f) g fatty acid/100 g neutral fats

(g) 9.8% milling degree

4.3. Composition of lipids. The proportion of FFA, NF and F in TL is similar in outer layer, nucleus and entire kernel (Table XV). However, differences in fatty acid composition of lipid fractions are apparent. Unsaturated acids -18:1 and 18:2- are responsible for the differences. They account for nearly 80 per cent of total fatty acids. Oleic acid is more abundant in outer layer than in nucleus both in FFA and NF fractions; the reverse happens with linoleic acid.

4.4. Chemical characteristics of lipids. (Table XVII). Acid values^(*) ranged between 40.8 and 44.9, the latter corresponding to the outer layer. Despite the high values obtained, fat acidity^(**) of the entire kernel was lower than the maximal value suggested for rice with little or no deterioration (205). This was not the case with the outer layer, the fat acidity of which exceeded such value. In the sample examined, acid values^(*) of lipids from the outer layer and the nucleus were 44.9 and 40.8 respectively. A higher acid value for lipids of the outer region of the kernel was also found in previous studies (See Part II). Lipids from undermilled rices had higher acid value than those from well milled rices (215). As it concerns wheat, higher values for bran lipids than for flour lipids have been reported (206).

Saponification values (Table XVII) of lipids in outer layer and entire kernel were quite similar: 192-193. These values are within the range of data reported in the literature for the fat of bran, brown rice and germ (5). Ali et al. (51) reported that the S.V. of fats in rice bran and brown rice are practically the same (188.3 and 186.6 respectively). In contrast with this, Yamasaki (45) reported that the fats of unpolished rice and of rice bran differ in S.V. from that of polished rice.

The iodine value of lipids of entire kernel (102.1, Table XVII) was within the range of values reported in the literature for lipids from milled and brown rices (33) (51) (71) (207). That of lipids from the outer layer was significantly higher (120.9) but comparable to that reported for lipids from rice polishings (209). In corn (210) and wheat oils (212) (213) similar values have been found. The higher I.V. of lipids of outer layer as compared with nucleus is in agreement with data published by Lugay and Juliano (33) for bran and milled rice, but is in contrast with those of Yamasaki (45) for milled and brown rices. It is unknown if these discrepancies are due to varietal differences or to different previous history of samples. Some authors have failed to find such differences (51) (208).

Peroxide values (Table XVII) were extremely low. Similar levels have been reported for brown (214) (92), under- and well milled rices (92).

Finally, the TBA test (Table XVII) did not show presence of malonaldehyde in any sample.

(*) mg KOH/g lipids (petroleum ether extract).

(**) mg KOH/100 g rice.

TABLE XVI. Chemical composition of milled rice: comparison of outer layer, nucleus and entire kernel. V.B: Lipids, lipid fractions and free fatty acids in rices of different milling degree.

	Undermilled rice (a)			Milled rice (b)		
	Entire kernel	Outer layer(c)	Nucleus	Entire kernel	Outer layer(c)	Nucleus
Total lipids (d)(e)	1.18	5.28	0.71	0.70	2.24	0.52
Free fatty acids(e)	0.29	1.05	0.20	0.15	0.44	0.12
Neutral fats (e)	0.73	3.52	0.42	0.43	1.54	0.31
Phospholipids (e)	0.16	0.72	0.09	0.10	0.25	0.09
Free fatty acids(e)(f)	14:0	Tr	0.01	Tr	Tr	Tr
	16:0	0.07	0.24	0.03	0.10	0.02
	16:1	Tr	Tr	Tr	Tr	--
	18:0	Tr	0.02	Tr	0.01	Tr
	18:1	0.08	0.37	0.04	0.14	0.03
	18:2	0.13	0.39	0.08	0.19	0.07
	18:3	0.01	0.02	Tr	0.01	Tr
	20:0	-	Tr	-	Tr	-

(a) 7.7% of milling

(b) 12.0% of milling

(c) 10% of the milled kernel

(d) Chloroform:methanol (2:1) extractable lipids

(e) g/100 g rice, dry basis

(f) The number to the left of the colon denotes the length of the carbon chain, and the number to the right of the colon represents the number of double bonds in the fatty acids.

TABLE XVII. Characteristics of lipids from outer layer, nucleus and entire kernel

	Entire kernel	Outer layer(a)	Nucleus
Acid value (b)	42.0	44.9	40.8
Saponification value (b)	192.8	193.6	-
Ester value (b)	150.8	148.7	136.6
Iodine value (c)	102.1	120.9	96.2
Peroxide value (d)	0.52	0.70	0.31
TBA value (e)	0	0	0

(a) 5% of kernel weight

(b) mg KOH/g lipids

(c) g iodine/100 g lipids

(d) meq. peroxide/kg lipids

(e) According to Tarladgis et al., (166)

General comments

As a result of the heterogeneous distribution of constituents within the rice kernel, the outer layer of milled rice greatly differs in its chemical composition from the nucleus (Figs. 7 and 8). The entire kernel shows intermediate values.

The unusual composition and properties of the outer layer become evident from the examination of the diagrams. Starch is the major constituent of rice, as well as of outer layer and nucleus. Along with amylopectin, amylose is the component predominant in amount in the milled entire kernel. As such it has been considered when investigating the chemical factors determining the characteristics and properties of milled rice. However, a different situation occurs in outer layer. Although amylopectin still is the major constituent, protein occupies the second position, it being almost two-fold more abundant than amylose. Proteins account for a fifth of the outer layer's weight. This certainly is a high proportion which demands to be taken into account.

As to the other constituents, none occurs in quantities greater than 0.75% in the entire kernel (Fig. 7). In contrast with this several constituents exceed this quantity in the outer layer: non reducing sugars (3.52%), neutral fats (3.52%) and free fatty acids (1.05%). These levels are actually remarkable. Non reducing sugars are about seven-folds the proportion found in the entire kernel and 35 folds that in the nucleus. More than 60 per cent of total sugars of rice are located in the outer layer.

Free amino acids, although in small proportion in relation with other rice components, occur in high levels. The 41 mg N/100 g rice found in the outer layer are about four folds the level in the entire kernel and account for about 40 per cent of total amino acids in milled rice. The occurrence of both amino acids and sugars in relatively high levels at the outer layer, suggests a greater possibility of the rice to certain deterioration reactions -such as non enzymatic browning- than previously thought.

Finally, the phospholipids, which in the entire kernel are less than 0.2%, in the outer layer amount to 0.72%. Being in mind their possible surfactant properties, their location in peripheral layers might be of interest in connection with water treatment of milled rice.

It is certain that differences in the chemical composition between the outer layer and the entire kernel are not so remarkable in well milled rice as in undermilled rice, nevertheless, they are highly significant too. On the other hand, it is certain that if a thinner outer layer is considered, differences become greater.

Consideration of the outer layer of the kernel appears to be a promising way for acquiring a more actual knowledge of the characteristics and properties of rice. The outer layer is responsible in a major part for the kernel behavior. Its importance becomes of major significance when -besides its unusual chemical composition- it is borne in mind that during milling, abrasion destroys the cell organization of the kernel surface and the kernel constituents become in intimate contact. The natural stability is lost and the opportunity of the constituents to react with one another is highly increased. In addition, other factors such as the microflora -concentrated on the surface- and the direct contact with environment-atmospheric oxygen, for instance- contribute to more accelerate reactions.

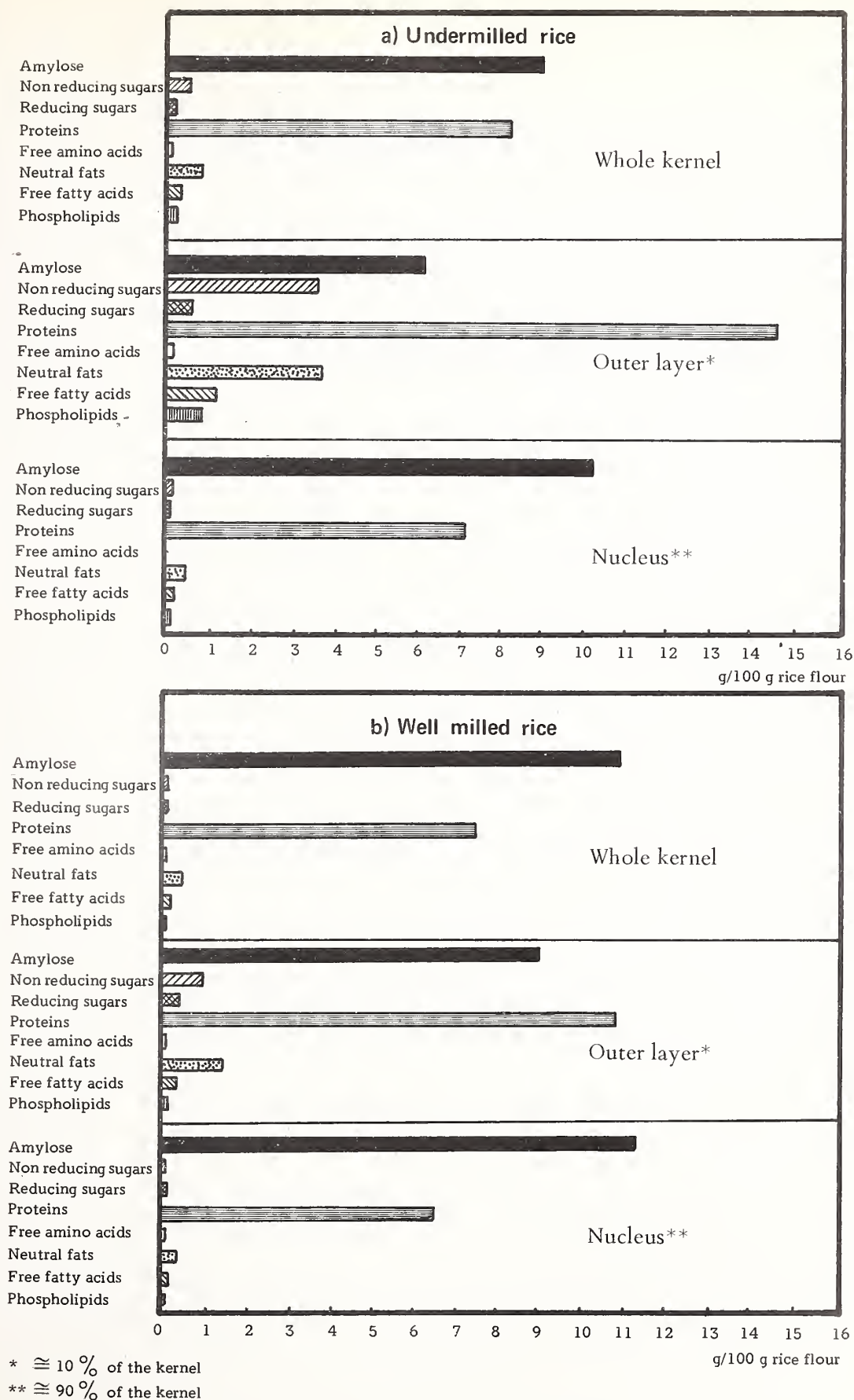


Fig. 7-8 .- Chemical composition of rice: comparison of outer layer, nucleus and whole kernel

II. BASIC STUDIES ON AGING OF MILLED RICE

INTRODUCTION

It is a usual practice to store rough rice after harvesting. Milled rice obtained from freshly harvested paddy is not suitable for consumption. Storage brings about changes in the chemical composition and properties of the kernel allowing the rice to be conveniently processed and safely consumed. Storage involves important problems. The harvested cereal must be conditioned to assure a good stability. Microorganisms, insects and rodents, causing large losses of material, must be controlled. Prevention of deterioration has requested most of the effort and a major part of the investigations on storage has been directed to develop a satisfactory and effective technology. Due to it, storage as a phenomenon has not been adequately studied.

Most of the information available on storage changes of rice concerns to rough rice, undoubtedly because this form of rice has been the most widely used for storing the cereal due to its better keeping quality. However, even for rough rice, fundamental knowledge of the chemistry of rice storage is meager.

Although storage of rice as paddy is preferable from the viewpoint of keeping quality, it involves some disadvantages of which bulkiness is the most important. For commercial and bulk storage and transport, milled rice is more economical than paddy. In fact, increasing quantities of milled rice are moving into the international market. For short-term storage processed rice is actually preferred. Commercially milled rice shows good keeping quality during short-term storage.

Storage at room temperature brings about changes in the physical and chemical characteristics of rice which modify the cooking quality, processing characteristics and nutritive value of the cereal. These changes may be limitative in the storage life of rice. Need for a more complete fundamental knowledge of rice storage is evident. The better understanding of the chemistry of storage should contribute to develop improved safety methods of storage, new or improved processes such as artificial rice improvement, and, in general, a more complete knowledge of the raw product to be processed.

LITERATURE REVIEW

Present review covers up to 1964. More recent papers are included in the Section "Results and Discussion". The work is focused on storage of milled rice, the subject of present research project. Information on storage of rough rice, brown rice and modified rices is also considered, although in a limited extent. Data for these rices constitute a source of basic knowledge, helpful for comparative purposes, but they concern to a substrate of different chemical stability, reaction systems and interactions.

In reviewing the published information on the effects of storage on the characteristics and properties of milled rice, the following topics are dealt with in separate sections: I. Rice quality. II. Physical and chemical characteristics of rice. III. Chemical composition of rice.

I. EFFECTS OF STORAGE ON THE QUALITY OF RICE

1. Keeping quality

In long term storage, milled rice is considered as a perishable product. In practice, storage is not longer than one year; in Spain it generally is shorter than six months.

The keeping quality of rice depends on environmental conditions and on the rice itself. Susceptibility of rice to deterioration is closely related to milling degree. The high lipid content of the surface layer, along with the enzymes and microflora there, favour unstability. Milling brings about remarkable changes in the chemical composition of outer layer, and, therefore, a substrate more or less liable to deterioration remains. Rough rice is protected by the hulls. Brown rice deteriorates faster; bran damage during shelling treatment accelerates the changes (214). Rao et al (92) reported the following data on the storage life of rice samples of different milling degree: brown rice^(x), 7 months; undermilled rice, 12 months; milled rice, 13 months. Fieger and Williams (216) also reported better keeping quality for milled than for brown rice.

As milled rice keeps well at room temperature, it allows for leaving out a cold storage which would make this popular and fairly cheap food excessively dear^(xx). However, storage at room temperature may bring about deterioration. It may cause considerable loss of product, and lowered quality, specially when appropriate precautions are not taken against high temperatures, not only in granaries and mills, but also in ships transporting the grain.

Moisture is another factor to be taken into account. It is a critical parameter. Along with temperature, it controls or enhances insect infestation and microbial growth, and has a decisive influence on biochemical reactions leading to deterioration and other changes in rice.

Intergranular atmosphere is another important factor influencing chemical and biochemical reactions of stored rice. Microorganisms and particularly insects are effected. Air-tight storage of cereals is a well known practice (226)(227)(228)(229)(230). It has been reported (217) that tastes of clean polished and 70% polished rice, stored for six years with 12% M.C. in air-tight containers, were merely as good as those of new rice. Nevertheless, hermetic storage of milled rice may bring about fast development of off-flavors.

As mentioned above, milled rice is not stored in practice longer than six-eight months. However, it has been published (218) that, under favourable conditions and adequate aeration, it can be stored up two years. Jagoe (219) also reported that undermilled rice stored in sacs in ventilated rooms, kept well for 20 months. Data from various authors (220)(221)(92) for milled rice stored in non ventilated non hermetic rooms show lower keeping quality.

(x) Stone-shelled

(xx) Cold storage is considered of interest in Japan (224)(225)(278)(279)

Susceptibility of rice to insect infestation varies with the degree of milling. Khan (222) made an study of the populations of store insects and the damage caused by them, during storage, to different grades of polished rice. According to this author, "grains milled twice and thrice are less attacked by insects and suffer less damage. Both of them keep well for longer periods than grains hand-pounded or milled once". Brown rice has been found to be more susceptible to insect attack than white, polished rice (231)(232). As quoted by Tamada (231), "Fraenkel and Brewett (233) reported from their study of several insects in stored products that all the species they observed seem to require vitamins of the B-group". As it is known, brown rice has higher thiamine and niacin content than white rice. "Balzer (234) stated that nearly all rice pests prefer brown to milled rice. Kunike (235) found that 100 individual rice weevils produced in 3 months 476 progeny in untreated husked rice, 15 in polished rice, and 15 in glazed rice".

The number of insects and the predominant species vary with storage conditions. Tanada (231) reported that "where the moths were more abundant and destructive, the rice was stored in semi-darkness, under a higher mean temperature and lower but more uniform relative humidity (warehouse A). The higher and more open conditions (warehouse B) appeared to be unfavorable to the moths" and favorable to the rice weevil, *Sitophilus oryza*, which was the insect of major importance. Higher total insect infestation was also found in rices in warehouse B (232).

Information on the changes of microflora during milled rice storage and their influences on the properties of rice are scanty, in spite of the importance that microbiological data have in evaluating rice as food. As it concerns storage, such information might be helpful to interpret chemical and physicochemical changes.

Most of the papers published concern, as it is usual in storage problems, to rough rice. The microflora occurring on and in the grain at harvest time evolves and changes during storage. In stored rice, molds, yeasts, actinomycetes and bacteria have been found in different number, it varying with the origin, previous history of the sample and obviously, storage conditions. Types of microflora predominant in stored rough rice are: *Pseudomonas*, *Bacillus* and *Aerobacter* among bacteria, and *Alternaria*, *Helminthosporium*, *Curvularia*, *Fusarium* and *Aspergillus* and *Penicillium* (the latter two particularly prevalent in latter stages) among fungi. That storage molds are largely confined to the two latter genera has been shown by several authors (236)(237)(138).

Milling brings about changes in microbial population of rice, both in number and types. Iizuka has investigated the changes of microflora during processing of Than rice (238) and Burma rice (239). Comparing unhulled and hulled samples of one year's old rice, he found lower number of moulds and of bacteria in the latter. "Of the species of bacteria Yellow *Pseudomonas* predominated and *Bacillus* was relatively less" (238). Removal of the husk generally brought about decrease in *Penicillium islandicum*, *P. citrium*, other *Penicillia*, *Aspergillus* (mainly *A. glaucus* group, *A. versicolor* group and white *Aspergillus* and other moulds than *Penicillium* and *Aspergillus*, and increase in yeasts and bacteria; the number of kernels not infested with internal microflora increased too (238)(239). Fungi in the unhulled and hulled seed had been previously studied by Del Prado and Christensen (237): *Aspergillus* (*A. glaucus* comprised 80% of the total) and *Penicillium* made up the bulk of the count of unhulled rice; also present were *Curvularia*, *Fusarium*, *Alternaria*, *Cladosporium* and *Streptomyces*. "Internal infection of seed itself, as distinguished from invasion of the hull" was shown as fungi grew from more than 50% of the dehulled kernel. "The principal molds

isolated from the caryopsis (brown rice), in order of prevalence, were Aspergillus (A. glaucus and A. niger), Curvularia, Penicillium and Fusarium. (In this connection, it is of interest to quote that Baldaci and Corbetta (240) in a mycological study carried out with thirty-three sample rice seeds stored in warehouse in Italy, found that the caryopsis which had been deglumed in the course of threshing were highly infected by Penicillium, Aspergillus, Mucor and Rhizopus and therefore represent potential centres for the beginning of fermentation).

When passing from husked rice to polished rice, similar trends to those commented above for the stage unhusked-husked rice were reported by Iizuka (238)(239).

"A comprehensive study on rice, carried out at the Southern Regional Research Laboratory (241) indicated that milled rice produced in the Southern parts of the United States in 1954 was not normally infected internally by fungi, bacteria, yeasts or actinomycetes. "The percentage of all the surface-sterilized kernels that were free from contamination was 99.1%. Ninety seven percent of the kernels which were water-washed only for the removal of surface- contaminating microorganisms were free from fungal contamination". "No Aspergillus or Penicillia species were found in water-washed samples" "None of the kernels treated with silver nitrate carried bacteria; among fungi were Alternaria, Phyllosticta, Cladosporium and Epicoccum species; practically no Aspergillus or Penicillium species were found. One culture of a Helminthosporium is cited.

Iizuka (238)(239) reported that the characteristic microflora of normal new unpolished rice in (Thailand and Burma) consists mostly of chromogenic Pseudomonas(*) and fluorescent Pseudomonas(*); the rest consist mostly of Aerobacter(*), Micrococcus, Brevibacterium (*), and molds such as Helminthosporium and Alternaria. Presence of mycelia of H. oryzae in infected rice (in U.S.A.) was demonstrated by Fazli and Schroeder (242) in various parts of the seed including the endosperm.

The work of Iizuka (238)(239) includes a comparative study of the microflora of polished samples of old and new rices stored as polished rice. This is an usual practice in Thailand. "In the freshly polished (one or two weeks before analysis) new crop rice, a great difference was recognized from the case of freshly polished old crop rice, the former giving a smaller number of non-infected kernels but a markedly high number of bacteria, about 60-80, mostly comprising Yellow Pseudomonas while the former gave only from 10 to 20 of bacteria. As to moulds found in freshly polished new crop rice, very few Aspergillus and Penicillium, and instead rather abundant Mucorales, Alternaria, Curvularia, Cepharosporium, etc. were detected. In contrast with this in the about one year old polished rice (from previous crop) Aspergillus predominated among moulds; Rhodotorula was rarely found among yeasts; the majority of bacteria was of the Bacillus megatherium; Streptomyces (consisting of S. diastaticus, S. Leben, Stessel and Keitt, S. carifonicus, S. albus, S. intermedium, etc.) in new and old polished rice were found and it was noticed that some of these gave considerably poisonous function when mice were fed with food containing rice with the strain grown on it". Unfortunately, it should be emphasised that the new and old rice samples studied were from different stock and crop and the original microflora at harvest time was not considered.

(*) In this connection it is of interest to quote the following papers (243)(244)(245)(246) (247)(248).

2. Eating quality

It is known that during storage some desirable changes in the properties of rice take place before deterioration. Primo et al (249)(250) made an organoleptic evaluation of the changes in white milled rice (13% M.C.) packed in linen bags and kept in drums at room temperature six months. The extent of changes was measured in terms of cohesiveness and overall acceptability of the cooked samples. Old rice was less sticky and had a higher acceptance than new rice. Decreased cohesiveness due to storage of rough or brown rice (even to drying) has been reported by several authors (135)(136)(138)(28)(92)(251)(96)(252).

Changes in the eating quality of milled rice during storage are influenced by holding conditions. Irwing (253)(254) reported that milled rice stored in a dry room either in sacs or jars scored higher than that stored in a refrigerator or a freezer. According to Tani et al. (224), the cooking qualities of white rice stored below 15°C were almost similar to that of rice when the storage was started. Maintenance of original high cohesiveness -sticky cooked kernels- is known to be one of the reasons for cold storage of rice in Japan.

Color and odor are two important characteristics in evaluating rice for consumption. The miller is aware of the difficulty in obtaining the same whiteness degree of milled rice from old and new stocks (it apart from fungal or other microbial discoloration of the kernel). In highly milled rice, color takes a long time to change. Yasumatsu and Moritaka (11) did not find significant changes after storing milled rice of about 13% M.C. at 9°C or at room temperature during six months. No information is available on the effects of storage on color of cooked milled rice. Although judging from the small changes in the raw samples, they should be expected to be non important.

II. EFFECTS OF STORAGE OF MILLED RICE ON PHYSICOCHEMICAL CHARACTERISTICS OF THE KERNEL.

Physicochemical characteristics of milled rice have been studied extensively in order to determine their relationship with the behavior of rice in cooking and processing (see Part III). In spite of the existing evidence for changes in these properties during storage, information on them is limited and, in general, there is not a critical evaluation of the significance of these changes in the value of the physicochemical characteristics for determining rice behavior.

2.a, Water absorption. Treatment of milled rice with hot water brings about changes in the weight and volume of the kernels, which, according to some authors, are associated with eating quality. Storage of milled rice affects the rate of water absorption and the volume of the watertreated rice. The changes depend on storage conditions. Yasumatsu et al (11) studied representative varieties of soft and hard quality rice, polished at 90% yield, packed in Kraft paper bags and stored at 9°C, 85% R.H., and at room temperature, for six months. Weight and volume increase by cooking in boiling water slightly decreased at 9°C, whereas little change on slight increase were noticed in samples stored at room temperature. Differences between cold and room storage effects on water absorption of milled samples from rice stored as paddy have also been reported (28). The pattern of changes appears to be common for all rices although quantitative differences occur among varieties (11)(249).

It should be pointed out that the temperature of cooking water is a factor to be taken into account. With boiling water, the water intake of old rice is higher than that of new rice, both for under- and well milled rices (92)(250). However, there is some evidence in support of the view that when cooking with water at 70°C the reverse happens. Such data have been reported for milled rice samples from stored rough rice (255)(96).

In this connection it should be pointed out that when rice is soaked in cold water or exposed to a damp atmosphere at 27°C, it absorbs an amount of water which is slightly higher in fresh than in old rice (251).

2.b. Solids in residual cooking water. Storage of milled rice brings about changes in the amount of solids passing to water during cooking; old rice yields less total solids than new rice (11)(249). Similar results were reported previously for rice stored as paddy (251)(255). The loss of solids in the residual liquids from cooking depends on storage temperature. The amount of solids and of solubilised starch is lower when milled rice is stored at room temperature than at 9°C (11).

The intensity of the blue color that the hot-water extracted starch gives with iodine ("blue test") is lower for old than for new milled rice (249). Although it appears to indicate an insolubilization of the linear component of starch, it is also possible that other factors be involved, such as the higher resistance of cell walls of old rice (133) and/or the insolubilization of the cementing proteins with storage (101)(92)(205).

2.c. Amylograms. The rheological characteristics of rice flour pastes change significantly with the age of milled rice. Old rice yields pastes of higher viscosity (256). Similar effects have been found during storage of rough rice (96)(255)(259)(224).

Knowledge on the effects of storage conditions on the amylogram characteristics of rice is scanty. It is known that high temperature levels during storage enhance the changes. Data for milled samples of Caloro, Rexoro and Bluebonnet rice varieties, stored at + 37.6°C, + 1.1°C and room temperature, showed that the hot paste viscosity increases faster when rice is stored at higher temperatures. Rices stored at + 1.1°C did not practically change (257). Similar results were reported for samples of the Japanese varieties Koshiji-wase and Asahi, stored as milled rice in Kraft paper bags, at 9°C and room temperature for six months (258). Similar trends have been reported for rough rice (255).

It should be pointed out that Yasumatsu et al. (258) in experiments cited above, did not find any difference in the gelatinization temperature between rices stored at 9°C and at room temperature. In contrast with these, some changes have been detected during storage of rough rice when determining the gelatinization temperature by the birefringence end point temperature technique (138). After 45 days' storage of paddy rice, the BEPT'S of the milled rice samples showed a slight increase but with increasing storage this trend appeared to be reversed, BEPT values becoming consistently lower.

Little is known about the factors responsible for storage changes in the amylogram characteristics of rice. Kester et al (195), studying the changes in the properties of rice during maturation, found a parallelism between the changes in peak viscosity values and those in amylase activity; highest amylase values were found at points of lowest viscosity. This parallelism, however, does not seem, to hold during storage. Alpha- and beta-amylase activities of rough rice decrease during storage (96).

The role of lipids in the changes of amylogram characteristics of stored milled rice have also been a subject of investigation. In rice, as in other cereals, storage brings about increase of the free fatty acid content. Fatty acids are known to increase the viscosity of starch (260)(261)(262) and wheat flour pastes (261). The effects of free fatty acids on rice pastes have been studied by various authors (256)(258)(263)(264). "The addition of oleic acid to Rexoro rice in an amount equal to half the total weight of lipids present raised its viscosity by about 100 Brabender units. But this effect was inadequate to account for the observed viscosity increase of stored Rexoro (rough) rice. Caloro pastes showed an even smaller response to added fatty acid" (256). In this connection Yasumatsu et al (258) reported that the increase in amounts of free fatty acids during rice storage resulted in the increase in maximum viscosity of amylogram. In the same work, the cited authors reported that methanol extraction (by the Schoch's method (265), i.e., 85% methanol) brings about decrease gelatinization temperature, changes in the shape of the amylogram and, practically, eliminates the difference in maximum viscosity of amylogram as exist between rice stored six months at 9°C and that at room temperature. Moreover, the amylogram of defatted rice powder reverts to its original shape by addition of the extract. The effect of methanol extract is almost accounted for by its free fatty acid fraction, and the amount of which and not the kind of fatty acids contained, is the dominant factor in determining the shape of amylogram.

On the contrary, partial defatting of milled rice with ethyl alcohol at 50°C does not bring about significant changes in the amylogram, it in spite of decreasing to 50% the original fat content (266). Extraction effects on the cooking quality of rice were also small (266). Similar effects were previously reported for soaking rice in 95% ethanol (101).

Normand et al., (267) studying the effects of heat treatment of rice on its processing and organoleptic characteristics, found out that curing of milled rice brings about changes in amylogram characteristics of rice flour pastes: peak and set back viscosities increase. According to the cited authors "the results of these experiments showed that changes brought about in a few hours were similar in sensory characteristics to those brought about normally by prolonged storage. The nature of the chemical or physical changes (or both) effected by heat treatment and those brought about through natural aging appear to be different".

2.d. Alkali digestion. The behavior of white rice kernels to treatment with diluted alkali has been widely used to evaluate rice quality (see Part III later on). Storage of rice affects kernel response to alkali treatment (268): "Several of the long-grain varieties had lower values for spreading after six months of storage at $\pm 3.3^{\circ}\text{C}/-4.4^{\circ}\text{C}$, whereas some lots of the medium- and short- grain varieties had higher values for spreading and lower values for clearing after storage". The chemical or physical factors responsible for these changes are not known.

III. EFFECTS OF STORAGE ON THE CHEMICAL COMPOSITION OF RICE

1. Carbohydrates

1.a. Starch. Total starch content of milled rice does not change during storage under normal conditions provided that insect and mould infestations are absent. The same occurs in rough rice (28)(255). In mould infested rice, starch can decrease significantly. Gosh (269) studied changes in the acidity and starch content of rice grains during fungal deterioration. In one case, the surface borne-natural fungal flora were allowed to develop on rice grains; in a second case, surface-sterilized grains were inoculated individually with 12 different fungi. In each case, the grains were stored at 80, 90 and 100% relative humidity: acidity increased with an increase in R.H. and with the time of storage. The increase was more pronounced in undermilled rice than in polished rice. After storing 42 days, the loss in starch content at 100 R.H. ranged between 15 and 25% for three different varieties, at 90% R.H. between 7.5 and 16%, whereas at 80% R.H. the starch loss was insignificant. Losses were definitely higher in artificially inoculated samples than in cases of mixed fungal growth. *Mucor* species "*Aspergillus versicolor*" and "*A. oryzae*" produced more acidity and maximum deterioration of starch content of rice grain.

Although starch content of milled rice remains practically constant during storage, its physicochemical characteristics undergo changes: its iodine combining capacity and its limiting viscosity increase with storage time (250). In contrast with this, decreased specific viscosity in alkaline solution of the starch after paddy storage has been reported (251). On the other hand, the latter authors found an increase in the iodine combining capacity. It should be noted that in this work (251) the studied new and old rices were from different harvests.

1.b. Starch fractions. As far as it is known data on changes in amylose and amylopectin content of milled rice with storage time are not available. During storage of rough rice no change (255) or slight variations (138) have been reported.

There is some evidence in support of the view that changes in some of the physicochemical characteristics of the starch fractions, as well as in the high and low molecular weight subfractions of the latter take place during storage (250). Increase of the iodine affinity of amylose has been reported during storage of milled (250) and rough rice (251).

In connection with storage changes in amylose content, it should be of interest to ascertain the influence that the storage modified iodine combining capacity of the linear fraction has on the results obtained using analytical methods based on the iodine-amylose blue color.

1.c. Sugars. "Alpha- and beta-amylases attack starch of grain and grain products during storage, converting it into dextrins and maltose". However increase in sugar contents not always takes place; "conditions that favor starch decomposition usually favor respiratory activity also, so that the sugars are consumed and converted into carbon dioxide and water. Under these conditions which usually occur at moisture levels of 15% or more, the grain

losses both starch and sugar and the dry weight decreases". "At higher moisture levels, however, active carbohydrate fermentation may occur" (270).

Qualitative and, especially, quantitative changes in sugars have been related to changes in keeping and cooking qualities of rice during storage. As compared with other carbohydrate constituents—such as starch (219)(271)(28) and amylose (255)(138)—, sugars are a sensitive parameter to follow storage changes. In general, in rough (135)(139)(271)(28)(272) as well as in brown rice (273)(272)(224)(225)(274), reducing sugars increase and non reducing sugars decrease during storage. If holding conditions are severe, reducing sugars decrease also with prolonged storage. Under mild storage conditions (M.C. less than 14% and room temperature or below, changes are small (271)(28).

Significant changes in sugars take place also during different technological processes—such as curing (276), parboiling (275)— and storage of parboiled rice (137).

It should be pointed out, however, that there is a lack of information regarding changes in sugars during storage of milled rice.

2. Nitrogen compounds

2.a. Total (TN) protein (PN) and non protein nitrogen (NPN). Data for under- and well-milled rice stored one year in gunny bags under room conditions (22.2° – 32.6°C and 35 – 80% M.C.), indicate that TN remains practically unchanged (92). On the other hand, Jagoe (219) reported previously that there was a drop in protein content of undermilled rice after three months storage, which showed recovery after seven months storage, but due to loss of starch as a result of insect damage and to decomposition of the fat; this protein recovery was, therefore, not real but relative. Significant losses have been reported after prolonged storage (220).

Data discriminating PN and NPN changes during storage of milled rice have not been reported. During ripening the ratio PN/TN increases and that of NPN/TN decreases (277).

2.b. Protein fractions. Variations in characteristics and properties of proteins during storage of milled rice have been reported. Nitrogen soluble in 3% sodium chloride solution decreases (92). N rendered soluble by digestion with pancreatin decreased during storage of raw-under- and well-milled rice (92). Storage of milled rice for long periods (two to ten years) brings about decreased protein digestibility and increased biological value (220).

In rough and brown rice similar trends have been found (92)(220). Decrease in solubility and in digestibility by proteolytic enzymes has been reported in other cereals (wheat, corn) (270). Moreover, nitrogen containing compounds soluble in dilute alkali of rough rice decrease (111) and water soluble nitrogen of brown rice decreases after reaching a peak (274).

2.c. Free amino N. There is not information available on changes during storage of milled rice. During storage of brown rice, amino acid N decreases (274)(84)(280); Tamura and Izumi (274) found a significant increase in amino acid N previous to a sharp decrease. During storage of parboiled rice, free amino acids decrease also (137)(80).

Data on changes in free individual amino acids during storage of rice have been reported by various authors (77) for rough rice, (84)(280) for brown rice, and (77)(80) for parboiled rice). According to Parihar (77) alanine, threonine, glutamic acid, lysine and histidine decrease during storage of rough rice; cystine was detected only after 2 year storage. Aspartic acid and glutamic acid decrease markedly compared with the other acids during storage of brown rice; leucine, asparagine, and cystine increase (84) (280). According to (80) the main loss during storage of parboiled rice corresponds to asparagine, methionine, cystine, glutamic acid, and alanine.

3. Enzymes

Data on the changes in enzymatic activities during storage of milled rice are limited. The existing information for various forms of raw rice (rough, brown and milled rice), indicates that in general, enzyme activities decrease with storage time. The work by Lozsa and Koller on changes during storage in the thiamin content and in the peroxylase and catalase activity of rice in Hungary (281) shows this trend; the authors reported: "In rice samples stored in polished or in ground state peroxidase and catalase activities disappeared only after 3-4 years of storage.

On storage the catalase and peroxidase activities of brown and rough rice decrease also (95). In this connection it is of interest to quote the results of Lozsa and Koller (281): "In rice stored as unhusked peroxidase and catalase activities run parallel, and their changes showed an annual fluctuation, i.e. the maxima of activities appeared always in April, the minima in October".

As quoted by Sreenivasan (28) the activities of alpha- and beta- amylase decrease as ripening advances. On storage the activities of the amylases of rough rice decrease (28) (96)(95). Similar results have been reported for brown rice (92). As noticed by Hogan (96), there are two aspects of the amylase picture which should be borne in mind: the activity of the amylase activity itself and the susceptibility of the rice starch. The cited author investigated the action of added alpha-and beta-amylase on the starch of stored rough rice, and reported that "the starch present in the aged rices becomes more susceptible to the attack of added alpha-amylase, reaching a maximum" and decreasing later on. Similar behavior -although delayed- showed the starch for the action of introduced beta-amylase.

Finally, decreased activities have been reported for other enzymes such as lipase (95), dehydrogenases, and glutamic acid decarboxilase (282). Activity of the latter has been found to be a more reliable index than fat acidity of the viability of stored rice(282).

4. Lipids.

4.a. Total lipids. No marked change in fat content of milled rice has been reported during safe storage (92). Similar results have been reported for rough rice (255). However, Jagoe (219) found a decrease in oil or fat content of milled rice due to decomposition and insect attack. Tsuchiya and Kinomura (209) found that during the storage of rice polishings for 17 months, the content of oil decreased to 3-5%; no insect infestation was reported.

4.b. Lipid fractions. It is a well known fact that during storage of rice titrable acidity increase, as a result of liberation of free fatty acids by hydrolysis. Data on acidity are abundant, particularly concerning rice by-products (see later on). However, there is a lack of information on changes of the various lipid fractions during storage of milled rice. The only paper available is that of Yasumatsu and Moritaka (11). Data for lipid fraction before storage are not given. However, differences caused by storing polished rice (from April to September) at room temperature or at 9°C are reported. Results are as follows: Neither total extraneous lipids nor total fat- by- hydrolysis showed any difference with storage temperature. However, differences in lipid composition appeared: total phospholipids and neutral fat decreased, and free fatty acid fraction increased, changes being larger in rice stored at room temperature.

4.c. Fatty acid composition of lipids. As reported in the work cited above (11), major fatty acid composition of total extraneous lipids and of neutral fat fraction do not vary with storage temperature. Fatty acid composition of free fatty acid fraction changes; oleic acid content was higher and linoleic acid content was lower when rice was held at room temperature.

4.d. Chemical characteristics of lipids. Deterioration of rice lipids brings about loss of flavor, development of off-odors, color fading, loss of nutritive value and other changes affecting the acceptability of the product. Oxidation and/or fat hydrolysis are the basic causes of these changes. Fat hydrolysis (catalysed by the lipase naturally existing in the kernel and that from microflora) takes place very much more rapidly than protein or carbohydrate hydrolysis; due to it fat acidity has been used as an index of rice deterioration during storage. Reported acidity data are much less abundant for stored milled rice (92)(269) than for stored rough (136)(135)(139)(111)(285)(288) and brown rices (283)(284)(214)(92)(224). Development of oxidative changes in rough rice is not usual under normal storage conditions; low levels of peroxide or carbonyl compounds have been reported (283)(285)(135). However, oxidative deterioration has been found to take place in brown (214)(92), milled (92) and parboiled rices (92)(286)(137). Milled rice undergoes smaller changes.

^ Fat deterioration during storage is greatly influenced by milling degree and sanitary condition of the grain, as well as on holding conditions. After one year storage under same conditions of undermilled (7% of polishings removed from husked rice) and well milled rice (milled to 14-15%), Rao et al. (92) found that the acidity of fats were 56.2 and 39.0 mg KOH/g fat respectively. In brown rice, bran damage has an important accelerating effect on development of free fatty acids (214). Changes in acidity of milled rice are influenced by fungal deterioration. In one experiment reported by Gosh (269), the surface borne-natural fungal flora were allowed to develop on rice grains and also, surface-sterilized grains were inoculated individually with twelve different fungi. In each case the grains were stored at

80%, 90% and 100% R.H. Acidity increased with an increase in R.H. and with the time of storage. The increase was more pronounced in undermilled rice than in polished rice. Mucor species, "Aspergillus versicolor" and "Aspergillus oryzae" produced more acidity. The effects of moisture on the microflora and formation of free fatty acids have also been studied in bran (287).

Under inadequate holding conditions and/or prolonged storage time, acidity may remain practically constant or decrease after reaching a peak (284)(209)(286)(288).

PLAN OF WORK

As shown throughout the Literature Review, rice, whether paddy or milled, raw or processed, does not remain unchanged with time. Rice does age; its cooking properties, processing characteristics and nutritive value change naturally and progressively. Changes do not necessarily imply deterioration; they may take place well in advance to the development of off-odors, loss of flavor or color fading. In fact, adequate storage brings about desirable changes in the properties and characteristics of rice. The phenomenon of aging is complex in nature. Rice is a heterogeneous substrate with a great variety of enzymatic activities. Moreover, rice is not an isolated system; bacteria, molds and yeasts are usual contaminants. Undesirable or desirable effects resulting from the chemical reactions involved in aging largely depend on the environmental conditions, length of time and on the rice itself. Rough rice, brown rice, and milled rice behave differently during storage. Due to all this and to the lack of sufficient systematic and comprehensive research on the subject, aging still is a not well understood phenomenon. This situation is more regrettable when it is realized that aging occurs inevitably when rice passes from harvest to consumption through the normal channels of processing and distribution.

A major difficulty for obtaining a more complete knowledge of aging lies on the fact that chemical changes under ordinary storage conditions are slow and small, being difficult to be measured by presently employed techniques. Recent advances in our knowledge of the distribution of the chemical constituents (see Part I) have shown that the outer layer—whether of the husked, undermilled or well milled grain—differs remarkably in chemical stability, reaction systems and interactions from the nucleus. Due to its unusual composition, remarkable changes are expected to occur at the outer layer. However, they will be unnoticed—because of the diluent effect of the more stable nucleus—unless the outer and the inner regions of the kernel be studied separately. Discrimination of the outer layer is of interest also because this layer plays a major role in determining cooking and processing qualities of rice, and contains a great part of the vulnerable nutrients of the kernel.

As stated previously, the primary objective of this investigation is to obtain information on the organoleptic, physicochemical, and biochemical changes in milled rice during storage. A second purpose is to develop basic knowledge of the interrelationships between chemical constituents and physical characteristics as affecting processing, cooking and

eating characteristics of rice. In this connection data on concomitant changes in quality and composition are expected to afford a substantial contribution. Although studies on rice quality are dealt with in a later section (see Part III), their changes during storage are reported here.

The plan of work includes^(*):

I. Effects of storage on the organoleptic characteristics of milled rice: 1.- Odor, 2.- Color, 3.- Cohesiveness, 4.- Overall acceptability.

II. Effects of storage on physicochemical characteristics of milled rice: 1.- Water absorption, 2.- Residual cooking liquids, 3.- Alkali test, 4.- Amylograms, 5.- N index, 6.- SH and SS indices, 7.- Intergranular atmosphere.

III. Effects of storage on the chemical composition of milled rice: 1.- Carbohydrates: 1.1.- Starch, 1.2.- Amylose, 1.3.- Sugars, 2.- Nitrogen compounds: 2.1.- Total, protein, and non-protein content, 2.2.- Protein solubility fractions, 2.3.- Sulfhydryl and disulfide contents, 2.4.- Free amino nitrogen, 3.- Enzymes: 3.1.- Alpha-amylase, 3.2.- Beta-amylase, 3.3.- Protease, 4.- Lipids: 4.1.- Total lipids, 4.2.- Lipid fractions: neutral fats, free fatty acids and phospholipids, 4.3.- Fatty acid composition of each three fractions, 4.4.- Chemical characteristics of lipids: acid, saponification, ester, iodine, peroxide and TBA values.

Storage changes in every item are investigated in the outer layer, the nucleus and the entire kernel of milled rice.

IV. Effects of storage on the microflora of milled rice: 1.- Bacteria and mold and yeast counts, 2. Types and proportion of microorganisms.

The work comprises three storage experiments carried out in successive years. Besides rice variety, the following variables have been studied: a) milling degree -from 7.6% to 12.6%-, b) moisture content -from 12.9% to 15.7%-, and c) temperature -from -20° to +35°C.

EXPERIMENTAL

MATERIALS

1964-65 Storage experiment

Americano 1600 rice -a short-grain variety- was selected for this study. The sample was harvested late in September at a moisture content of 28 - 26%, wet basis. Sun-drying, the usual practice in this area, was the procedure employed to bring the rice to 13.5% moisture content. In November, approximately 100 Kg of rough, rice were brought to our Laboratory

(*) Additional work on storage changes is reported in Part III.

and preliminary tests to determine the two appropriate milling degrees for storage studies were performed. The milling process of rice was carried out in a dehuller of the firm "Imad", Valencia, Spain, followed by whitening at an experimental mill manufactured by the firm "Torrejón", of Valencia. The broken kernels, as well as the chalky, green and unripened ones were removed with appropriate sizing devices. Aliquants from each sub-lot (that is, from each degree of milling) were temporarily stored in several cylindrical iron containers, on screen trays, over different saturated salt solutions, at 15°C, until approximately the moisture content of the rice came to equilibrium with the storage atmosphere within the drums. After a week the rice samples were removed with moisture contents of 13.3% and 14.7%, and transferred to glass bottles with push-in plastic stopper, and distributed in the different conditions of temperature. For storing rice in an atmosphere periodically renewed with fresh air, a climatic cabinet was designed and built which allowed us to maintain in it the temperature and moisture conditions within very narrow limits ($\pm 0.2^\circ\text{C}$ and $\pm 2\%$ relative humidity). The temperature control was automatically checked by two contact thermometers serially set up, one of them working as a safety device. The cabinet was equipped with a Laboratory-made device for conditioning the fresh air previous entering into the cabinet; a measuring orifice, previously calibrated, allowed us to control the amount of air; a recording thermohygrograph, set up inside the cabinet provided a continuous measurement of both moisture and temperature. A very simple device, with plastic hoses, allowed us to manipulate inside the cabinet or remove rice samples without changes in the inner atmosphere. Samples packed in sealed glass bottles and requiring $\pm 25^\circ\text{C}$ also were stored in this cabinet. Rice samples and storage conditions are given in Table XVIII.

Table XVIII. - Rice samples and storage conditions: 1964-65 experiment

Rice samples	Milling degree	Moisture content (%)	Temperature ($^\circ\text{C}$)
II-1	undermilled (7.6%)	13.3	+ 5
II-2	"	13.3	+ 25
II-3	"	14.6	+ 5
II-4	"	14.6	+ 25
II-5 (x)	"	14.6	+ 25
V-1	milled (12.6%)	13.4	+ 5
V-2	"	13.4	+ 25
V-3	"	14.7	+ 5
V-4	"	14.7	+ 25
V-z	"	14.7	- 20

(x) Stored in an aerated cabinet.

1965-66 Storage experiment

Balilla x Sollana rice variety -short kernel- was used in this study. Samples were prepared as the previous year except that they were dried at room temperature using partially dried air or humidified by spraying water to obtain the desired moisture contents. Samples with intermediate moisture levels did not need any treatment. Storage conditions used are given in Table XIX.

Table XIX.- Rice samples and storage conditions: 1965-66 experiment

Rice samples	Milling (%)	M. C. (%)	Storage	Temperature (°C)
II-A. 1	7.7	12.9	Hermetic	+ 5
II-A. 2	"	"	"	+ 25
II-A. 3	"	"	"	+ 35
II-A. 4	"	"	"	- 20
II-B. 1	"	13.7	"	+ 5
II-B. 2	"	"	"	+ 25
II-B. 3	"	"	"	+ 35
II-B. 4	"	"	"	- 20
II-X. 2	"	"	Aerated	+ 25
II-C. 1	"	15.6	Hermetic	+ 5
II-C. 2	"	"	"	+ 25
II-C. 3	"	"	"	+ 35
II-C. 4	"	"	"	- 20
IV-A. 1	12.0	13.1	Hermetic	+ 5
IV-A. 2	"	"	"	+ 25
IV-A. 3	"	"	"	+ 35
IV-A. 4	"	"	"	- 20
IV-B. 1	"	14.2	"	+ 5
IV-B. 2	"	"	"	+ 25
IV-B. 3	"	"	"	+ 35
IV-B. 4	"	"	"	- 20
IV-X. 2	"	"	Aerated	+ 25
IV-C. 1	"	15.5	Hermetic	+ 5
IV-C. 2	"	"	"	+ 25
IV-C. 3	"	"	"	+ 35
IV-C. 4	"	"	"	- 20

1966-67 Storage experiment

Balilla x Sollana rice variety was used. Samples were prepared by spraying water to obtain the desired moisture contents. Rice samples and storage conditions are given in Table XX.

Table XX.- Rice samples and storage conditions: 1966-67 experiment

Rice samples ^(*)	Milling degree (%)	Moisture content (%)	Temperature (°C)
A. 1	9.8	13.0	+ 5
A. 2	"	"	+ 25
A. 3	"	"	+ 35
B. 1	"	14.3	+ 5
B. 2	"	"	+ 25
B. 3	"	"	+ 35
C. 1	"	15.7	+ 5
C. 2	"	"	+ 25
C. 3	"	"	+ 35

(*) Hermetic storage

1967-68 Storage experiment

Rough rice samples were dehulled and milled as described above, packed in air-tight bottles and stored. Rice samples and storage conditions are given in Table XXI.

Table XXI.- Rice samples and storage conditions: 1967-68 experiment

Variety	Milling degree (%)	Moisture content (%)	Temperature (°C)
Bomba	≈ 8	13.2	+ 25
Balilla x Sollana	≈ 8	14.0	+ 25
Gema	≈ 8	12.0	+ 25

METHODS

I. EFFECTS OF STORAGE ON THE ORGANOLEPTIC CHARACTERISTICS OF MILLED RICE.

1. Odor

Raw samples were rated for odor using the following scale: none 9; doubtful, 7; perceptible, 5; moderately strong, 3; and strong, 1.

2. Color

Raw samples were rated for color using the following scale: white, 9; creamy, 7; light brown, 5; brown, 3; and dark brown, 1. Color measurements were also carried out using a Hunter Color and Color Difference Meter Model D-25. The instrument was adjusted with a standard color reference plate ($L = 93.6$; $a = -1.0$; $b = 2.3$).

3. Cohesiveness

Cooking of samples was carried as usual at this Laboratory (309). A trained tasting panel evaluated cooked rices for cohesiveness using the score card reported by Batcher et al. (295). The balanced incomplete block design (310) was used for comparison of samples at the panel.

4. Overall acceptability

Samples were rated using the hedonic scale (296). Quality criteria were defined in a previous paper (309).

II. EFFECTS OF STORAGE ON PHYSICOCHEMICAL CHARACTERISTICS OF MILLED RICE

1. Water absorption

The procedures described by Halick and Kelly (289), and Halick and Keneaster (290) were used for determining water absorption at 70°C and 100°C , respectively.

Water absorption during cooking at optimum cooking time was determined after preparing the samples as for sensory evaluation (309) and measuring water content by the two-stage method (146). Moisture content of original samples was deducted and results given as grams of absorbed water per 100 g raw rice, dry basis.

2. Solids in residual cooking liquids

They were determined by evaporating in a water bath an aliquot of the residual liquids from cooking the samples at their optimum time, and then drying in an oven at $102 \pm 2^{\circ}\text{C}$ up to constant weight. Results are given in grams per 100 g raw rice, dry basis.

3. Alkali test

The method of Little et al (268) was followed.

4. Amylograms

A Brabender amylograph, USK.4 model, Duisburg, Germany, was used. The procedure followed was described in a previous paper (125).

5. N index

It was determined as described previously (74).

6. SH and SS indices

a) Reagent: The solvent system used for the estimation of SH groups contained 200 g of acetamide, 500 ml. of 95% ethanol, 100 ml of 0.5 M-ammonium nitrate in 2.5 M-ammonia and was made up with distilled water to 1000 ml. This is a slight modification of the solvent medium used by Bloksma (297) and adopted by Axford et al. (143). For the estimation of the SS groups, 10 g of sodium sulphite in aqueous solution were added before making up to 1000 ml.

b) Procedure: A 10 g sample of raw milled rice kernels was transferred, previous counting the number of kernels, into a 125 ml bottle with push-in plastic stopper containing 50 ml of the solvent reagent. Nitrogen having passed previously through an ammonia wash-bottle, was used for eliminating air in the head space of the bottle. The stoppered bottle was shaken on a mechanical machine for one hour. Then, the extract was filtered through a stainless steel wire gauze (40 mesh/inch) into the titration vessel. SH or SS groups in the extract were estimated amperometrically with silver nitrate according to the procedure described by Axford et al. (143). Results are reported as $\mu\text{eq.}$ of SH or SS per kernel $\times 10^5$. These are the uncorrected values of the SH or SS indices. Surface corrected indices were calculated by the formula:

$$\text{Corrected values} = \text{Uncorrected value} \frac{1}{S_t}$$

where S_t is the surface in cm^2 of the ellipsoid of revolution, given by

$$S_t \simeq 8.89 b \sqrt{a^2 + b^2}$$

$b = \frac{(b' + b'')}{2}$ being the arithmetic average of the smaller semiaxes and a the larger semiaxle of the generatrix ellipse.

A more simple though less accurate calculating procedure is by the formula:

$$\text{Corrected value} = \text{Uncorrected value} \frac{1}{\sqrt[3]{\left(\frac{P}{n}\right)^2}}$$

where P is the total weight of the sample and n the number of kernels. (It is assumed that kernel density is practically constant for all varieties).

7. Intergranular air

7.1. CO_2 . Gas chromatography analysis of intergranular air were made with a Perkin-Elmer 116-E; column were: Molecular sieve 5-A Ca-Al-silicate 62SO91 type, 2 m, 0.5 cm \varnothing , for N_2 and O_2 and silica gel (Jo) 62SO92 type, 2 m, 0.5 cm \varnothing , for CO_2 ; column temperature 50°C .

7.2. Germinative capacity of rice kernels. It was measured by the 2:3:5 - triphenyl tetrazolium chloridemethod as outlined by Bloch (291). Simple germination tests were also carried out; kernels were placed on moist paper incubated at room temperature for 5 days, and the number of seedlings counted.

III. EFFECTS OF STORAGE ON THE CHEMICAL COMPOSITION OF MILLED RICE

Methods used were described in Part I (see pages 20 to 23).

IV. EFFECTS OF STORAGE ON MICROFLORA OF MILLED RICE

For microbiological analysis, ten grams of rice were mixed with 90 cc of sterile distilled water in a sterile Waring Blendor cup for two minutes; serial dilutions of this suspension were plated for aerobic bacteria in tryptone glucose agar (Difco) and for moulds and yeasts in Czapeck medium (Difco). The cultures were incubated at 37°C and 30°C, respectively. The same cultures were used for plate count and isolation of microorganisms. Identification of microorganisms was carried out by the usual techniques (292)(293)(294).

RESULTS AND DISCUSSION

I. EFFECTS OF STORAGE ON ORGANOLEPTIC CHARACTERISTICS OF MILLED RICE.

1. ODOR

Data from 1965-66 and 1966-67 storage experiments are shown graphically in Fig. 9. Off-odors developed during storage of milled rice at a rate depending on three inter-related factors: degree of milling, moisture content and temperature. Data from the 1965-66 experiment (Fig. 9a) show that, at +35°C, odor development was detected at the first sensory evaluation session (3rd month) in all samples except the well milled rice with 13.1% M.C. At +25°C off-odors were clearly perceptible at the third month in some samples. Progressive staling continued throughout the experiment, and at the end of it off-odors were present in all rices. The well milled rices with 13.1% and 12.4% M.C. did not become objectionable at this time. At +5°C perceptible changes were detected at the 3rd month in undermilled rice with 15.6%. After ten months off-odors were present in all the undermilled rices, and in the well milled sample with highest moisture content. At -20°C there was no

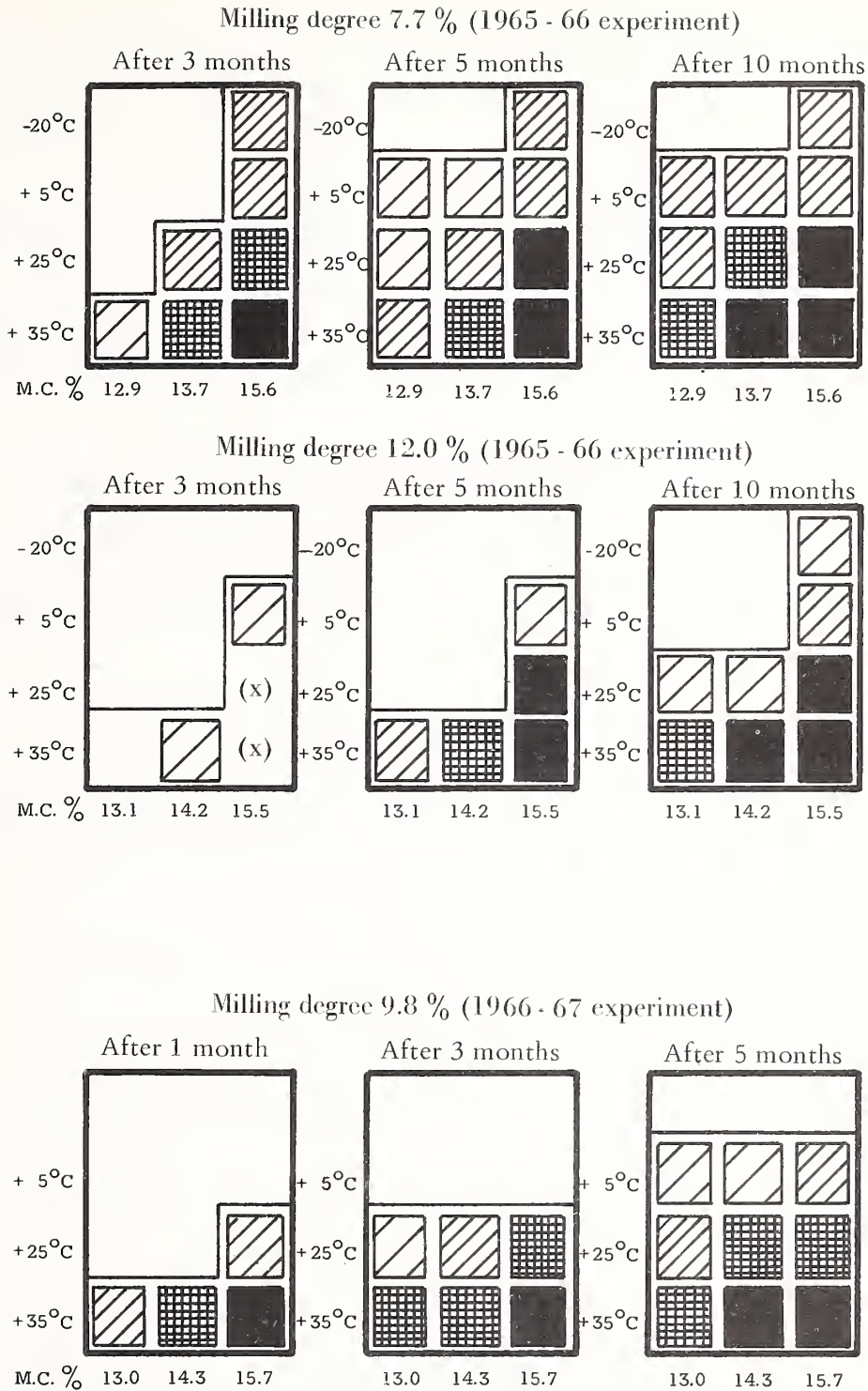
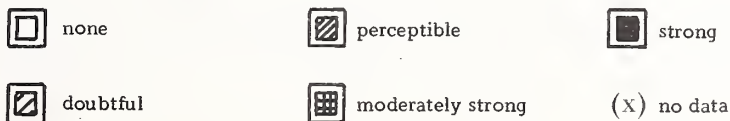


Fig. 9.- Development of off-odors during air-tight storage of milled rice



change of odor; undermilled rice with 15.6% M.C. was an exception. Results from the 1966-67 experiment were in fair agreement with the previous year's data. It will be noted that organoleptic tests carried out after one month storage (Fig. 9b) showed the early appearance of off-odors in samples held at $\pm 35^{\circ}\text{C}$.

That well milled rice keeps better than undermilled one has been reported previously (92), however the cited authors found that undermilled and milled rices (7% and 14-15% of polishings removed from husked rice, respectively) did not develop rancid odor during one year storage in gunny bags at room conditions. The major difference between this and our experiment is that our samples were stored in air-tight bottles. Nevertheless, one sample stored in an aerated cabinet (see Fig. 12) deteriorated before three months storage.

Our results on the influence of temperature upon development of off-odor are in agreement with the data reported by Yasumatsu et al. (321) (298) (299) for cooked samples from rices stored in non hermetic containers. The cited authors reported that "when polished rice was stored at $+9^{\circ}\text{C}$ (in Kraft paper bags, relative humidity 80%) no change was observed in the flavor of rice (cooked samples) within six months (298), but remarkable flavor deterioration was observed in polished rice stored at room temperature (298) and when stored two months at 40°C (299)".

2. COLOR

Rice samples were inspected for color by sensory evaluation. In addition, color was measured with a Hunter Color and Color Difference Meter. Results obtained during the 1965-66 and 1966-67 storage experiments are given in Tables XXII and XXIII.

Data of the 1965-66 experiment show that storage at $\pm 35^{\circ}\text{C}$ resulted in pronounced browning: at the 3rd month in undermilled samples with 13.7% and 15.6% M.C. and in well milled sample with 15.5% M.C.; at the fifth month browning was also present in undermilled rice with 12.9% M.C. and in well milled rice with 14.2% M.C. After ten months, all samples were discolored. Some of the well milled samples gave Hunter color values similar to those of undermilled rices. Storage at 25°C produced color changes within three months in undermilled rices with 13.7% and 15.6% M.C. and in well milled rice with 15.5% M.C.. At the fifth samples at the tenth month. There was one exception: well milled with 12.9% M.C.. At $\pm 5^{\circ}\text{C}$ and below, there were no significant differences in color during ten months of storage. Undermilled rice with 15.5% M.C. was the only exception.

Changes in Hunter color values generally followed the changes noted by the score panels. With storage time "L" values decreased, and "a" and "b" values increased. As indicated by the "a" and "b" values, browning involves a variation of the color towards the red and the yellow components, the latest showing the greatest change.

Visual inspection of the germ residual in some kernels allowed to detect changes in color earlier. Browning of the germ appeared even in samples stored at $\pm 5^{\circ}\text{C}$. In samples greatly deteriorated, staining apparently migrated from the germ to the starchy endosperm.

Table XXII. - Changes in color of milled rice during storage (1965-66 experiment)

Samples(1)	(1) Storage(2) time	Visual color										Triestimulus color factors (Hunter values)												"ΔE"		
		Kernel						Germ (3)				"L"			"a"			"b"								
		0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3	
II-A.4		7	7	7	7	7	7	6	5	71.3	71.3	70.9	-0.5	-0.8	-0.1	13.7	13.8	13.9	25.0	25.1	25.5					
II-A.1		7	7	7	7	7	7	6	5	71.3	71.2	71.6	-0.5	-0.4	-0.1	13.7	13.8	13.8	25.0	25.2	24.8					
II-A.2		7	7	7	6	7	7	4	4	71.3	71.0	71.2	-0.5	-0.1	0.0	13.7	14.1	14.4	25.0	25.5	25.5					
II-A.3		7	7	6	5	7	-	4	1	71.3	70.1	69.3	-0.5	+0.6	+0.8	13.7	15.6	16.5	25.0	27.0	28.2					
II-B.4		7	7	7	7	7	6	6	5	71.6	71.6	71.1	-0.5	-0.7	+0.1	13.8	13.8	13.7	24.8	24.8	25.2					
II-B.1		7	7	7	7	7	6	6	5	71.6	71.0	71.4	-0.5	-0.5	-0.1	13.8	13.9	13.8	24.8	25.4	25.0					
II-B.2		7	5	6	5	7	6	6	4	71.6	71.4	72.3	-0.5	+0.1	+0.1	13.8	14.0	14.4	24.8	25.1	24.5					
II-B.3		7	5	2	1	7	1	1	1	71.6	66.8	66.1	-0.5	+1.4	+2.0	13.8	15.8	16.8	24.8	30.1	31.2					
II-C.4		7	7	7	5	7	6	6	4	70.3	70.4	70.1	-0.3	-0.7	+0.1	14.4	14.4	14.0	26.2	25.9	26.3					
II-C.1		7	7	5	4	7	6	5	3	70.3	70.9	71.1	-0.3	-0.6	+0.1	14.4	13.8	14.2	26.2	25.4	25.5					
II-C.2		7	5	6	3	7	4	3	2	70.3	70.7	70.1	-0.3	+0.3	+0.4	14.4	14.2	14.6	26.2	25.8	26.5					
II-C.3		7	3	2	1	7	1	1	1	70.3	67.3	64.7	-0.3	+1.8	+3.4	14.4	16.4	17.9	26.2	30.0	33.1					
II-X.2		7	7	7	6	7	-	5	4	71.6	70.7	70.3	-0.3	-0.4	+0.1	14.4	14.0	14.8	26.2	25.7	26.4					
IV-A.4		9	9	9	9	7	6	6	6	77.7	76.9	76.6	-1.1	-1.5	-0.8	12.6	12.5	12.4	18.9	19.6	19.7					
IV-A.1		9	9	9	9	7	6	6	6	77.7	77.0	76.3	-1.1	-1.1	-0.8	12.6	12.5	12.5	18.9	19.5	20.1					
IV-A.2		9	9	9	9	7	6	6	6	77.7	75.7	76.5	-1.1	-0.8	-0.8	12.6	12.8	13.3	18.9	20.7	20.3					
IV-A.3		9	-	8	6	7	6	1	1	77.7	75.6	74.6	-1.1	-0.6	-0.1	12.6	14.6	16.0	18.9	21.8	23.4					
IV-B.4		9	9	9	9	7	6	5	5	76.8	76.6	76.4	-1.0	-1.4	-0.9	12.6	12.6	12.5	19.7	19.9	20.0					
IV-B.1		9	9	9	9	7	6	5	5	76.8	76.7	76.9	-1.0	-1.1	-1.0	12.6	12.6	12.6	19.7	19.8	19.6					
IV-B.2		9	9	9	8	7	6	5	4	76.8	76.6	76.6	-1.0	-0.7	-0.5	12.6	12.9	13.5	19.7	20.0	20.3					
IV-B.3		9	9	7	5	7	-	1	1	76.8	72.8	73.3	-1.0	+1.1	+0.5	12.6	15.9	16.3	19.7	24.9	24.7					
IV-C.4		9	9	9	9	7	6	5	5	75.7	76.1	76.0	-1.2	-1.4	-0.8	13.0	12.9	12.7	20.9	20.5	20.4					
IV-C.1		9	9	9	9	7	6	5	5	75.7	75.8	76.4	-1.2	-1.1	-0.6	13.0	12.7	12.9	20.9	20.6	20.2					
IV-C.2		9	7	7	6	7	-	3	2	75.7	75.8	75.9	-1.2	-0.3	-0.3	13.0	13.1	13.6	20.9	20.8	21.0					
IV-C.3		9	7	5	3	7	-	1	1	75.7	72.9	69.4	-1.2	+1.1	+3.6	13.0	15.9	17.8	20.9	24.7	29.1					
IV-X.2		-	-	-	8	-	-	5	5	76.8	75.8	76.0	-1.2	-1.0	-1.0	13.0	13.1	14.1	20.9	20.8	21.2					

(1) See Table XIX for identification. (2) Determinations were carried out at the beginning of storage (time 0), and after three (time 1), five (time 2), and ten months (time 3). (3) % kernels with germ; undermilled rice 42% and well milled rice 9%.

Table XXIII. - Changes in color of milled rice during storage (1966-67 experiment).

Rice Samples	Visual color						Triestimulus color factors (Hunter values)															
	Kernel			Germ(3)			"L"				"a"				"b"				"ΔE"			
	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2				
(1)(2)	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2				
A - 1	8	8	8	7	7	6	74.2	74.5	74.6	-1.5	-1.9	-1.6	13.0	12.8	12.9	22.4	22.2	22.1				
A - 2	8	8	8	7	7	6	74.2	74.4	74.6	-1.5	-1.9	-1.6	13.0	12.9	13.0	22.4	22.3	22.2				
A - 3	8	8	8	7	6	6	74.2	74.5	74.4	-1.5	-1.9	-1.6	13.0	13.7	14.2	22.4	22.6	22.9				
B - 1	8	8	8	7	7	6	74.1	74.2	74.8	-1.6	-1.9	-1.7	13.4	12.9	12.7	22.7	22.4	21.8				
B - 2	8	8	8	7	7	6	74.1	74.4	74.9	-1.6	-1.9	-1.6	13.4	13.2	13.2	22.7	22.4	22.0				
B - 3	8	7	5	7	3	1	74.1	73.4	71.8	-1.6	-1.4	-0.3	13.4	14.5	16.0	22.7	23.9	26.1				
C - 1	8	8	8	7	6	6	74.3	74.4	74.3	-1.6	-1.9	-1.6	13.2	13.0	13.1	22.4	22.3	22.4				
C - 2	8	8	8	7	6	3	74.3	74.8	74.8	-1.6	-1.7	-1.2	13.2	13.3	13.2	22.4	22.1	22.1				
C - 3	8	6	3	7	1	1	74.3	72.9	70.5	-1.6	-1.0	+0.6	13.2	14.8	16.5	22.4	24.5	27.4				

(1) See Table XX for identification

(2) Determinations were carried out at the beginning of the storage (time 0), and after three (time 1) and five months (time 2)

(3) Percentage of kernels with germ: 17%.

Data of the 1966-67 experiment were in agreement with the previous year's results. It will be noticed in Table XXIII that storage at $+35^{\circ}\text{C}$ resulted in color changes within the first three months in sample with 15.7% M.C.; a slight dullness was noted by some of the panel members in rice with 14.3% M.C.

Data of both storage experiments showed that degree of milling, moisture content and temperature are closely related factors influencing color stability. At $+5^{\circ}\text{C}$ or below, no color changes took place within 5 months in rices of milling degree ranging between 7.7% and 12.0% and M.C. between 12.9% and 15.6%. At higher temperatures ($\geq 25^{\circ}\text{C}$) color stability depended on the three parameters.

In this connection, a recent paper by Pelshenke (300) is of interest. This author reported that white rice held for one year under air, nitrogen, oxygen and carbon dioxide atmospheres, undergoes very little color changes at $+2^{\circ}\text{C}$ and $+20^{\circ}\text{C}$, but all samples developed a marked yellow color at $+35^{\circ}\text{C}$. As quoted in the Literature Review, Yasumatsu and Moritaka (11) did not find significant changes after storing milled rice at $+9^{\circ}\text{C}$ nor at room temperature during six months. In a related work on storage of rough rice (138), no changes in the color of milled rice were observed when the rough rice was stored with M.C. less than approximately 16% during six months at 21° - 26°C .

3. PALATABILITY CHARACTERISTICS OF COOKED RICE: COHESIVENESS AND ACCEPTABILITY.

Results from three storage experiments are illustrated in Figs. 10 to 12. The following conclusions are withdrawn from the 1965-66 (data Fig. 11): At -20°C and $+5^{\circ}\text{C}$ there were no significant changes. However, off-flavors were perceptible after ten months in all undermilled samples held at $+5^{\circ}\text{C}$ and consequently a slight progressive decrease in the overall acceptability should be expected this time hereafter. At $+25^{\circ}\text{C}$ cohesiveness decreased (panel test ratings increased), preference increased firstly but decreased afterwards, when off-flavors appeared. At $+35^{\circ}\text{C}$ rice became stale and rancid very soon. The reversal of the trend of changes was not detected.

Changes in under- and in well milled rices showed similar trends although the rate and extent of the latter were smaller. Despite the high degree of milling of the well milled rices, samples with the highest moisture content deteriorated rapidly when held at $+25^{\circ}\text{C}$ or at $+35^{\circ}\text{C}$.

Data from other two storage experiments (Figs. 10 and 12) confirmed this information.

It will have been noticed in Fig. 12 that undesirable rancid odor was clearly detected in aerated rice (sample 11-5) sooner than in air-tight stored rices. The deleterious effect of ventilation was confirmed by supplementary tests conducted with undermilled rice samples of three rice varieties- Benlloch, Balilla and Peladilla-. One part of each lot was wrapped with iron gauze, and another filled into glass bottles and sealed; and samples were stored at room temperature at the Laboratory and organoleptically evaluated at monthly intervals. Air-tight stored samples kept longer: development of typical rancid odors and bitter taste (suggesting lipoxidase attack on unsaturated fats) seems to be enhanced by atmospheric oxygen.

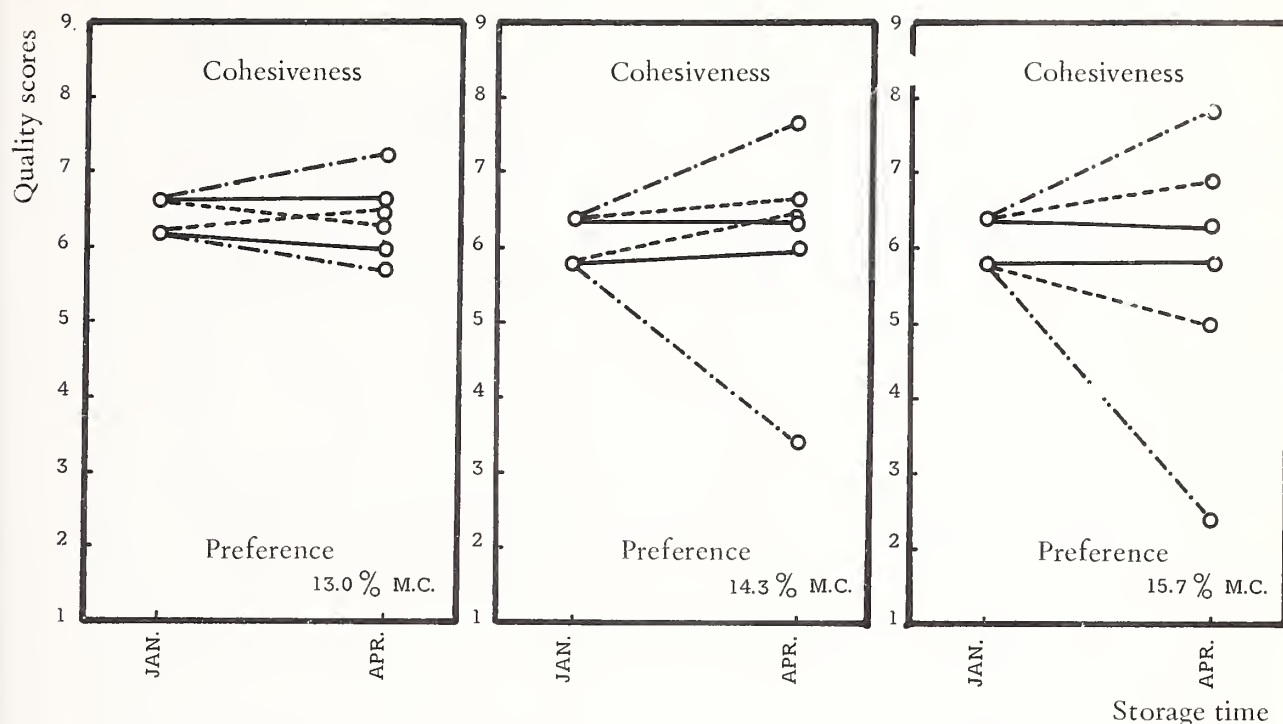
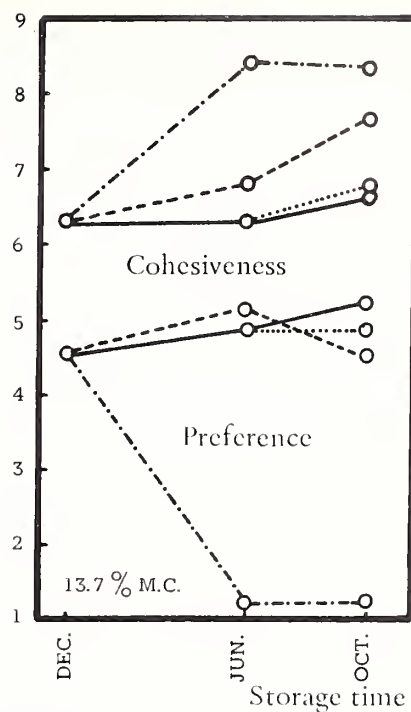
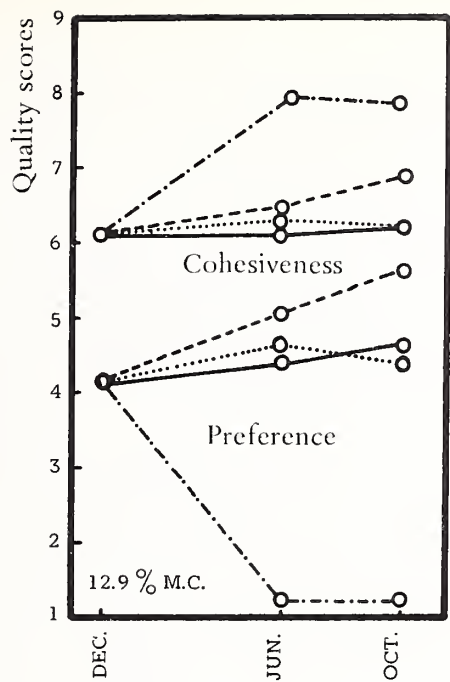


Fig. 10.- Effects of air-tight storage on the quality of milled rice (1966 - 67 experiment)

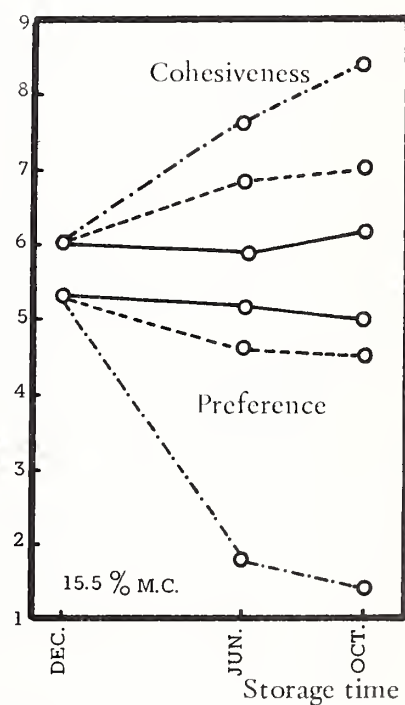
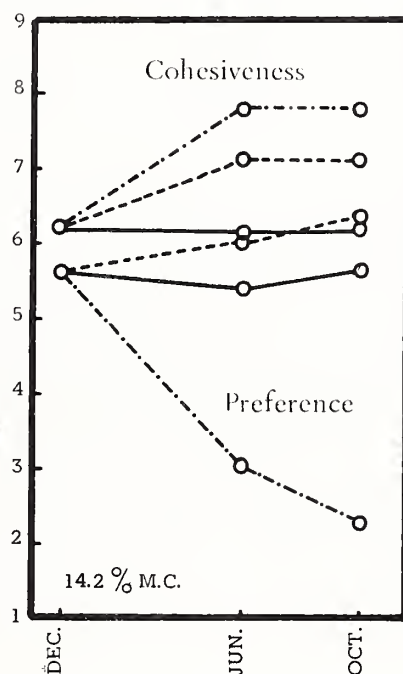
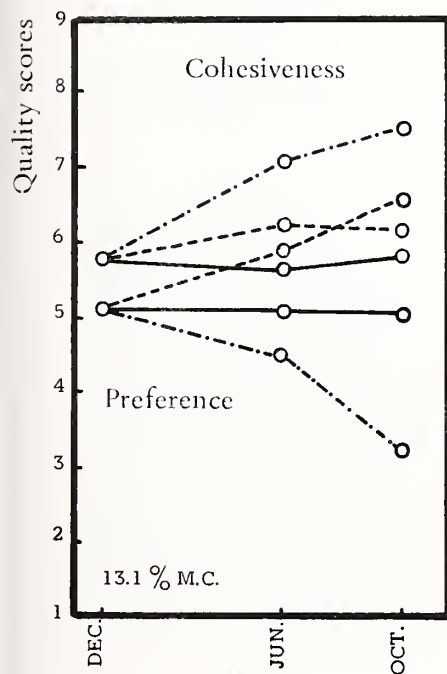
— at + 5°C
 - - - at + 25°C
 - · - · at + 35°C

It can be concluded from all experiments that: a) Quality improves during storage as a result of decreased cohesiveness of the cooked kernels but development of off-odors may counteract this effect resulting in a loss of quality. b) Changes are dependent on milling degree, moisture content and temperature. c) At +5°C or below quality is maintained practically unchanged.

In order to ascertain whether the trends of storage changes in the physical and organoleptic properties of rice are common for all varieties, the following experiment was carried out: 21 rice varieties, collected from main rice producing areas of Spain, were milled at the laboratory and an aliquot of each was packed in air-tight bottles and stored at +35°C, to be studied after an aging period of four months. The remaining part of each sample was used in characterising the rices before storage. Data on the characteristics of studied samples and on changes in color, odor, cohesiveness and overall acceptability are given in Table XXXIII-A. Although the extent of changes was small, results indicated a general trend of changes for all varieties. Similar conclusions are withdrawn from data reported by various authors (298)(11)(224)(225)(272)(253)(254)(249). As to the magnitude of changes, it appears that, in general, initial state of grain and storage conditions are more important than variety. It should be noted however, that there is some evidence (298)(324) that varietal differences in the rate of changes may occur. The latter authors reported: early season rice deteriorates more rapidly than normal rice in initial phases



a) Undermilled rice (7.6 %)



b) Well milled rice (12.6 %)

Fig. 11.- Effects of air-tight storage on the quality of milled rice (1965 - 66 experiment)

..... at -20°C - - - - - at $+25^{\circ}\text{C}$
 ————— at $+5^{\circ}\text{C}$ - · - · - at $+35^{\circ}\text{C}$

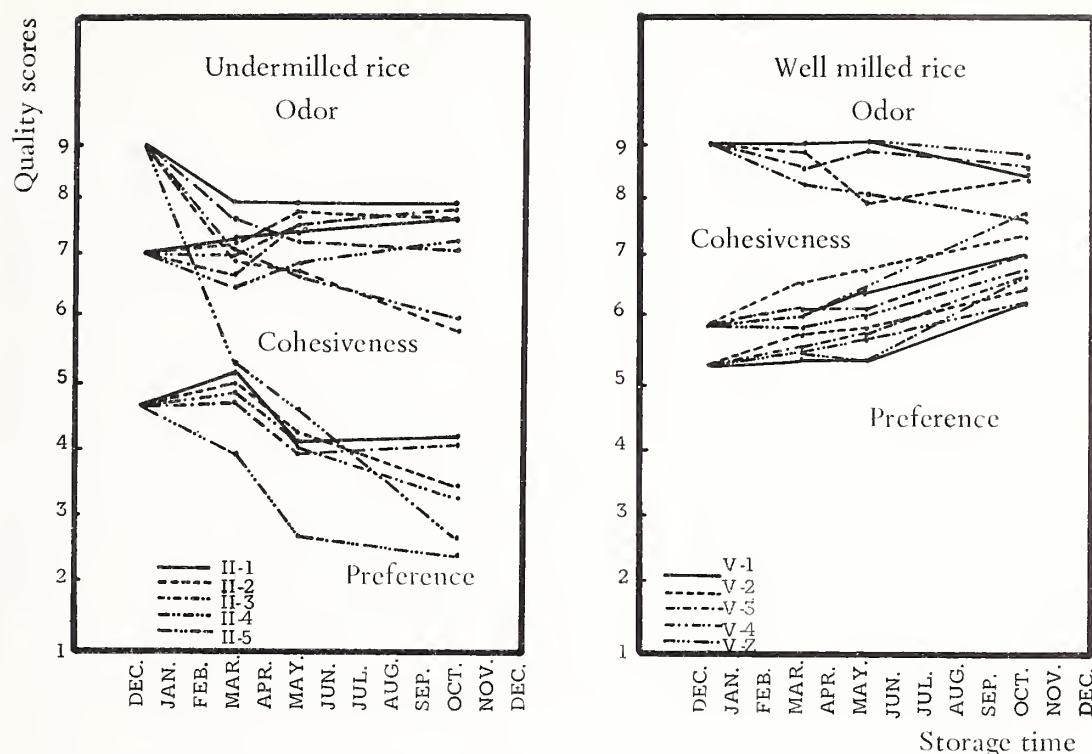


Fig. 12.- Effects of storage on the quality of milled rice (1964 - 65 experiment)

(See Table XVIII for identification of samples)

of storage; nevertheless in prolonged storage, differences are not appreciable.

The great many data gathered in the three consecutive storage experiments comprise samples with quite different milling degrees, moisture contents and temperatures. All these data have been plotted in Fig. 13 to show the variation of storage life of milled rice in relation to holding conditions. Four characteristics have been considered: a) infestation by insects, b) visual color -raw rice-, c) odor -raw rice- and d) preference or over all acceptability -cooked rice-. Boundaries of every zone in the graphs correspond to the first score lower than the acceptable level. It is interesting to note that: a) all samples were free of infestation, b) unless milling degree is high and moisture content low, prolonged storage at $+5^{\circ}\text{C}$ results in loss of acceptability and c) milling degree should not be neglected or undervaluated when storing milled rice; this parameter has a marked influence on the keeping quality of the cereal.

II. EFFECTS OF STORAGE ON PHYSICOCHEMICAL CHARACTERISTICS OF MILLED RICE.

1. WATER ABSORPTION

Data given in Table XXIV show that water absorption increased significantly during storage. These results were consistent with previous data for milled rice (321)

Table XXIIIA. - Storage changes in milled rice of different varieties.

Characteristics of samples										Physical characteristics raw rice.				Eating characteristics cooked rice.			
Sample no	Variety	Origin	M. C. %	Milling degree %	Protein content %	Fat content %	Color "L"			Odor	Cohesiveness	Overall acceptability					
							0	1	0			1	0	1			
1	Sollana	Calasparra	13.70	13.56	9.74	0.35	69.2	69.0	9	7	6.9	7.5	6.3	6.5			
2	Stirpe	Sevilla	13.72	13.89	9.59	0.34	68.6	68.5	9	8	6.1	6.5	5.8	6.1			
3	Bomba	Calasparra	13.30	9.16	9.54	0.42	65.4	65.3	9	7	8.2	8.3	8.0	7.9			
4	Frances	Tortosa	12.62	11.84	8.72	0.30	71.5	71.5	9	8	5.9	6.6	5.7	6.6			
5	Bomba	Tortosa	13.60	10.36	9.71	0.60	68.5	68.3	9	7	8.2	8.7	7.8	8.0			
6	Nex S	Sevilla	13.50	12.85	9.13	0.35	70.1	69.9	9	8	6.3	5.7	5.3	5.2			
7	Girona	Tortosa	13.65	11.89	8.42	0.42	67.2	67.0	9	8	5.6	6.6	5.2	6.4			
8	Bahia	Sevilla	13.40	10.54	7.26	0.40	67.5	67.3	9	7	5.8	7.0	5.6	6.1			
9	Gema	Sevilla	13.67	10.31	7.58	0.43	62.5	62.4	9	6	5.6	6.1	5.4	5.6			
10	B x S	Sevilla	13.20	9.32	8.95	0.49	70.1	70.1	9	7	6.3	6.3	5.6	5.7			
11	Sequal	Sevilla	13.35	9.67	9.09	0.40	65.1	65.0	9	6	5.8	7.2	5.8	6.0			
12	Dosel	Sevilla	12.65	11.49	7.85	0.47	67.4	67.3	9	7	5.2	6.7	5.3	6.5			
13	Matusaska	Tortosa	13.00	11.12	7.64	0.56	67.6	67.3	9	8	5.9	5.4	5.7	5.8			
14	Liso	Sueca	12.80	12.48	8.66	0.36	65.4	65.2	9	8	5.7	5.9	5.5	5.9			
15	Rafaelo	Sevilla	13.47	11.13	10.07	0.56	69.4	68.6	9	7	7.5	7.8	6.2	6.0			
16	Balillone	Sueca	13.15	9.68	6.96	0.46	65.0	64.8	9	8	5.7	6.6	5.5	5.6			
17	B x S	Sueca	12.85	12.53	7.50	0.37	70.2	70.1	9	7	5.6	7.0	5.2	5.7			
18	Sequal	Sueca	13.25	9.46	6.74	0.46	70.1	69.4	9	8	5.4	6.2	5.1	5.2			
19	Pegonil	Calasparra	12.15	9.67	7.41	0.30	70.5	70.3	9	8	6.3	7.3	5.9	6.7			
20	Balilla	Sueca	13.35	9.57	7.73	0.35	70.3	70.1	9	8	5.7	6.7	5.7	5.9			
21	Girona	Sueca	13.27	7.72	7.31	0.48	66.2	66.0	9	5	5.9	6.0	5.6	5.6			

(1) Determinations were carried out at the beginning of storage (time 0) and after four months (time 1).

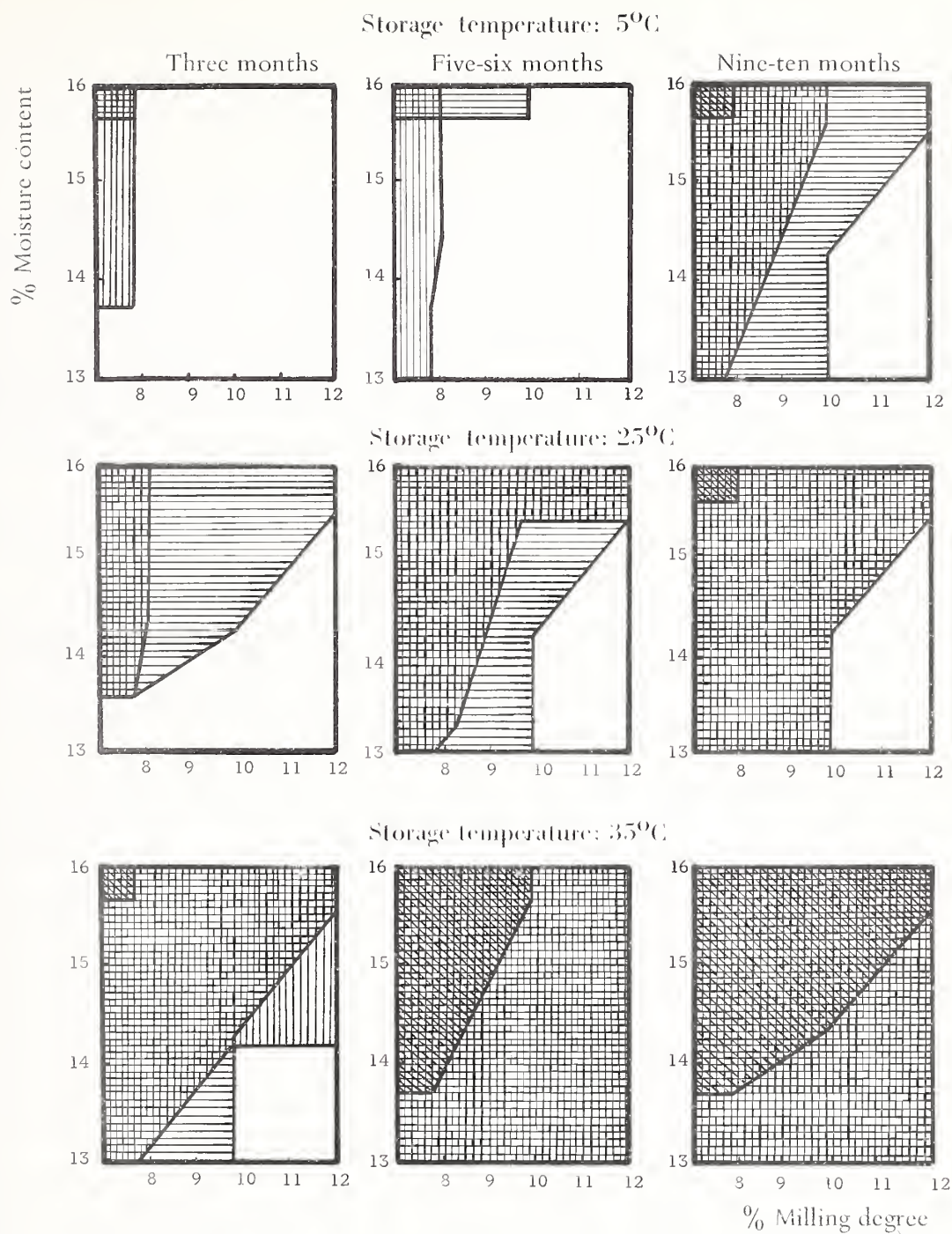


Fig. 13.- Keeping quality of air-tight stored milled rice: Influence of temperature, moisture content and milling degree.



Preference

Off-odors



Visual color

Infestation by insects

(92) (249), brown rice (224)(272)(225) and roughrice (272). It should be mentioned, however, that inverse results have been obtained when water absorption was determined by "cooking" the samples at 70°C (96) instead of in boiling water. Water absorption changes were accelerated by higher storage temperatures. Yasumatsu and Moritaka (11) also found that storage of polished rice in Kraft paper bags at 9°C resulted in lower weight increase than at room temperature.

Storage at +5°C resulted in increased absorption. In this connection, it has been reported however, that the ratio of the volume of cooked rice to that of uncooked rice does not increase when paddy is stored at 0°C during four months (28).

It is of interest to point out that despite the significant changes in water absorption, the water content of the cooked rice samples varied between very narrow limits: 73-75%. Samples stored at higher temperature showed the higher values but the surface of their cooked kernels appeared drier than in those of the fresh or less aged samples.

2. TOTAL SOLIDS IN RESIDUAL COOKING LIQUIDS

Data given in Table XXIV show that storage decreased the total amount of solids eluted during cooking, confirming previous results for milled (249)(11), brown (272)(224)(225) and rough rice (272)(251)(255). Samples held at +5°C did not show significant changes. In samples stored at +25°C slight decreases were observed, and at +35°C remarkable changes took place. High moisture contents also accelerate the changes.

Table XXIV.- Effects of storage on water absorption and residual solids.

Samples (1)	Storage time(4)	Water absorption (2)(5)				Residual solids (3) (5)			
		0	1	2	3	0	1	2	3
A - 1		256	269	284	291	6.4	6.6	6.7	6.6
A - 2		256	277	317	291	6.4	6.4	5.8	5.3
A - 3		256	283	307	304	6.4	5.0	4.9	4.6
B - 1		258	272	284	273	6.3	6.3	6.4	6.2
B - 2		258	269	302	-	6.3	5.7	5.7	5.3
B - 3		258	286	309	311	6.3	4.0	3.4	3.1
C - 1		262	270	291	287	6.1	5.8	6.3	6.4
C - 2		262	271	304	304	6.1	5.8	5.7	5.2
C - 3		262	288	302	288	6.1	3.4	2.9	2.6

(1) See Table XX for identification.

(2) g water/100 g rice.

(3) g/100 g rice.

(4) Determinations were carried out in February (storage time 0), April (time 1), July (time 2) and October (time 3).

(5) Samples were cooked to their optimum cooking time: A, 18.20 min.; B, 17.40 min.; C, 16.30 min. Changes during storage were smaller than one minute.

3. ALKALI TEST

Photographs in Fig. 14 show the effects of alkali treatment after two and four months of storage of under- and well milled rices (1964-65 storage experiment). As it can be seen, the alkali test does not establish significant differences among samples stored for different periods nor among samples held under quite different conditions.

Similar results were obtained with all the samples of the 1966-67 experiment. The effects of alkali treatment were quite similar for all the samples and periods (from January to October). A slight increase in disintegration was noticed from January to March in all samples; the magnitude of the change was practically equal for all rices, independently from moisture content and temperature. The changes reported by Little et al., (268) have not been confirmed.

The lack of significant changes is in contrast with the large modifications found in rice properties, both organoleptic and physicochemical.

4. AMYLOGRAMS ^(*)

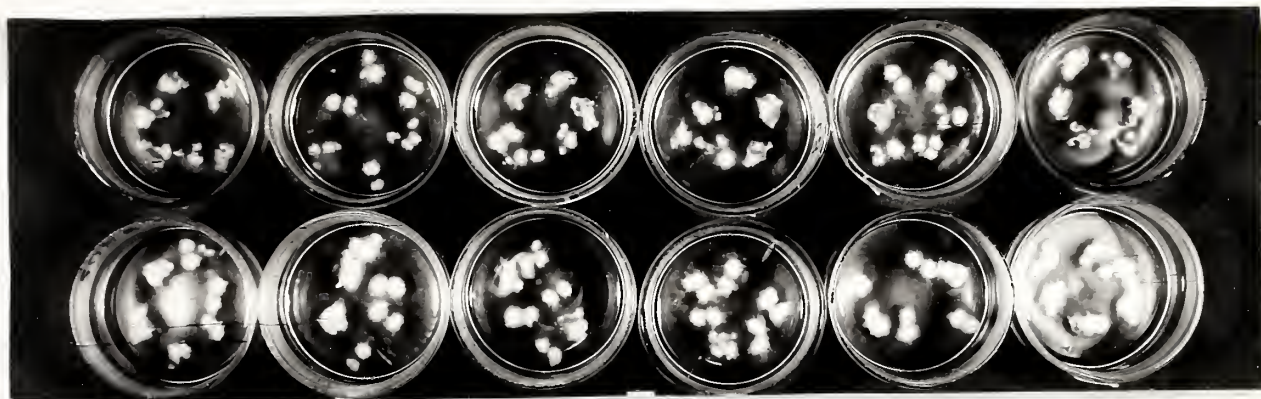
Data on the effects of milled rice storage on the gelatinization and pasting characteristics of rice flours are given in Tables XXV, XXVI and XXVIII, and are illustrated in Figs. 15 and 16.

4.1. Gelatinization temperature. Small changes (1° - 2° C) were observed during storage of under- and well milled rices in the 1964-65 experiment (Table XXV). A slight increase took place at the beginning of storage but for more prolonged time, the general trend was to decrease. Similar results were previously obtained by Schroeder and Halick (138) working with stored rough rice. There was not a correlation between gelatinization temperature and acidity (Table XXVII). Increased gelatinization temperature should however be expected as a result of fatty acids formation (260)(262)(258)(263)(11)(311).

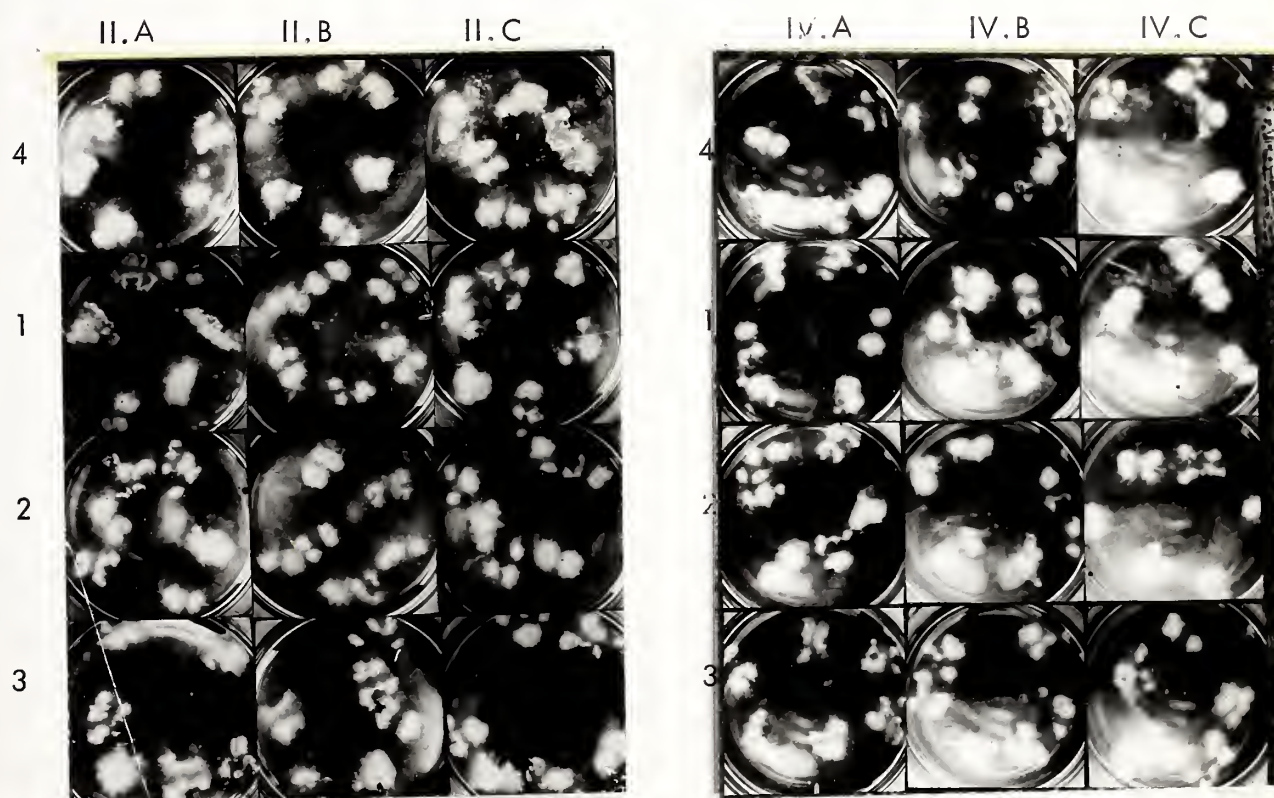
Gelatinization temperature has been related to the cooking characteristics of rice (289)(302) and, although there is some evidence that it is not correlated with the eating quality (303), it is being used in evaluating rice for various purposes (304)(305)(306)(307). Therefore, confirmation of storage effects was considered convenient. In the previous work, gelatinization temperature was determined with the Brabender Amylograph at a concentration of rice slurry of 45 g rice flour per 450 cc of water. In the confirmatory work the rice-to-water ratio was 100 g rice flour/400 cc water, as suggested by Halick et al. (308) for more precise results. The data obtained (Table XXVIII) were in agreement with previous data. There was not practically any significant change. Similar results were reported by Yasumatsu et al. (258); they did not find any difference in the gelatinization temperature between polished rices stored at 9° C and at room temperature.

4.2. Peak viscosity. Data from the 1964-65 experiment (Table XXV, Fig. 15) showed two different general trends of changes associated with the storage temperature. Samples stored at low temperatures ($+5^{\circ}$ and -20° C) showed an initial decrease, this trend appearing to be reversed with increasing storage time. On the other hand, samples stored at $+25^{\circ}$ C exhibited a constant increase since the beginning. Similar results have been reported by

(*) See also Part III.



a) Rice kernels of under- and well milled samples treated with dilute alkali in February 1966. Upper side, left to right: II.A.2, II.B.2, II.C.2, IV.A.2, IV.B.2, and IV.C.2. Lower side: duplicates. (See Table XIX for identification of samples).



b) Rice kernels of under- and well milled samples treated with dilute alkali in May 1966. (See Table XIX for identification of samples).

Fig. 14.- Effects of storage on dispersibility in alkali of milled rice.

Table XXV.- Effects of storage on amylogram of milled rice (1964-65 experiment)

Samples (1)	Storage time(2)	Characteristic points of the amylogram															
		Gelatinization temperature				Peak viscosity				Minimum viscosity				Viscosity at 50°C			
		0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
II - 1		85.5	86	85	-	800	735	735	-	660	645	635	-	1260	1235	1235	-
II - 2		85.5	85	84.5	-	800	775	805	-	660	675	740	-	1260	1300	1420	-
II - 3		85.5	86	84	85	800	635	640	780	660	575	540	640	1260	1160	1180	1260
II - 4		85.5	84	83.5	82	800	865	880	940	660	755	770	780	1260	1430	1485	1570
II - 5		85.5	86.5	86	82	800	800	835	870	660	715	750	740	1260	1430	1500	1300
V - 1		83.5	85	81	83.5	840	740	835	850	700	620	725	680	1280	1140	1300	1240
V - 2		83.5	82	83.5	82	840	1065	1075	1160	700	845	720	900	1280	1430	1510	1570
V - 3		83.5	85	84	84	840	730	730	750	700	635	630	630	1280	1150	1170	1180
V - 4		83.5	82	82.5	81.5	840	1070	1090	1120	700	880	930	940	1280	1460	1600	1590
V - 5		83.5	84.5	84	83.5	840	680	735	725	700	590	670	570	1280	1080	1170	1130

(1) See Table XVIII for identification.

(2) Determinations were carried out on January (storage time 0), March (1), May (2) and October (3).

Table XXVI.- Effects of storage on amylogram of milled rice (1965-66 experiment)

Samples (1)	Storage (2)	Characteristic points of the amylogram								
		Peak viscosity			Minimum viscosity			Viscosity at 50°C		
		0	1	2	0	1	2	0	1	2
II-A.4		680	660	650	560	500	530	960	1050	1060
II-A.1		680	720	710	560	550	560	960	1150	1150
II-A.2		680	900	1120	560	680	800	960	1320	1530
II-A.3		680	1260	1420	560	890	1060	960	1690	1900
II-B.4		640	580	620	530	540	500	1080	960	990
II-B.1		640	690	640	530	550	500	1080	1130	1030
II-B.2		640	1070	1100	530	710	750	1080	1400	1470
II-B.3		640	1300	1360	530	1000	1160	1080	1810	2200
II-C.4		800	720	600	660	560	470	1250	1160	990
II-C.1		800	740	780	660	560	560	1250	1180	1190
II-C.2		800	1140	1240	660	760	860	1250	1500	1720
II-C.3		800	1410	1270	660	1040	1120	1250	1860	2180
IV-A.4		790	-	850	660	-	680	1180	-	1260
IV-A.1		790	-	890	660	-	580	1180	-	1110
IV-A.2		790	-	1350	660	-	970	1180	-	1590
IV-A.3		790	1460	1570	660	1060	1240	1180	1770	2050
IV-B.4		820	-	730	670	-	660	1200	-	1130
IV-B.1		820	-	800	670	-	700	1200	-	1170
IV-B.2		820	-	-	670	-	-	1200	-	-
IV-B.3		820	1530	1460	670	1030	1260	1200	1800	2160
IV-C.4		1140	-	920	840	-	660	1440	-	1230
IV-C.1		1140	-	900	840	-	650	1440	-	1230
IV-C.2		1140	1240	1450	840	810	900	1440	1470	1640
IV-C.3		1140	1560	1020	840	1130	940	1440	1900	1230
II-X.2		640	890	1120	530	670	720	1080	1320	1400
IV-X.2		820	-	1280	670	-	870	1200	-	1480

(1) See Table XIX for identification.

(2) Determinations were carried out at the beginning of storage (time 0) and after three (time 1) and five months (time 2).

Table XXVII.- Changes in titrable acidity and pH during storage of milled rice.

Samples (2)	Storage time (3)	Fat acidity (1)			pH		
		0	1	2	0	1	2
II - 1		26.4	31.0	33.8	6.6	6.6	6.7
II - 2		26.4	41.2	77.2	6.6	6.7	6.6
II - 3		26.4	30.3	39.4	6.6	6.7	6.6
II - 4		26.4	43.1	88.2	6.6	6.4	6.5
II - 5		26.4	57.4	95.6	6.6	7.1	6.4
V - 1		6.7	13.4	12.0	6.4	6.6	6.5
V - 2		6.7	15.4	18.1	6.4	6.6	6.4
V - 3		6.7	10.3	13.8	6.4	6.5	6.4
V - 4		6.7	14.1	14.2	6.4	6.5	6.4
V - z		6.7	8.0	7.7	6.4	6.6	6.6

(1) mg KOH/100 g rice, dry basis. (2) See Table XVIII for identification. (3) Fat acidity: Determinations were carried out on December (time 0), January (1), March (2) and October (3); pH: January (time 0), March (1), June (2) and October (3).

Yasumatsu et al. (321) and Tani et al. (224).

Temperature appears to be the predominant influencing factor on the trend of changes. Neither the moisture content (within the narrow range studied) nor the degree of milling showed a definite influence. The influence of storage temperature on peak viscosity changes was also reported by Yasumatsu et al (321)(258). According to these authors polished rice stored at room temperature had higher peak viscosity than that held at 9°C.

Data from the 1965-66 experiment are given in Table XXVI. They were in good agreement with the results of the previous test. Two general trends of changes, associated to the storage temperature were confirmed: a) Samples held at low temperatures (-20° and +5°C) showed a slight decrease in peak viscosity after which exhibited a progressive increase. b) Samples held at high temperatures (+25° and +35°C) exhibited a constant increase since the beginning of the storage. The higher the temperature, the sharper the rate of the increase was.

Moisture content of rice appears to influence the changes of the samples stored at the highest temperature. Peak viscosity of rice held at +35°C increases until reaching a maximum and then decreases sharply. The rate of changes is closely associated to moisture level. This influence is not clear at lower temperatures.

The change in the peak viscosity trend seems to take place at higher levels of viscosity for the well milled rices than for the undermilled rices. In spite of the more advanced state of deterioration of the undermilled rices, their peak viscosity changes seem to be smaller. This appears to be particularly true in the samples of higher moisture content (i.e., samples IV-C.3 vs. II-C.3).

Table XXVIII.- Changes in gelatinization temperature during storage of milled rice (1966-67 experiment).

Rice samples (1)	Storage time (2)	Gelatinization temperature ($^{\circ}$ C)		
		0	1	2
A - 1		67.1	67.1	67.2
A - 2		67.1	67.1	67.1
A - 3		67.1	67.1	67.2
B - 1		67.1	67.2	67.1
B - 2		67.1	67.1	67.1
B - 3		67.1	66.9	68.0
C - 1		67.4	67.1	67.2
C - 2		67.4	67.5	67.4
C - 3		67.4	67.0	68.6

(1) See Table XX for identification. (2) Determinations were carried out at the beginning of storage (time 0), after 2 1/2 months (time 1) and after five months (time 2).

4.3.- Minimum viscosity. Paste viscosity decreased during the fifteen minutes period at constant temperature (92.5° C). Data from 1964-65 and 1965-66 experiments showed that minimum viscosity changed with storage time in a similar way to peak viscosity.

4.4.- Loss of hot paste viscosity (at 92.5° C). This parameter exhibited an irregular trend of changes, different in each of the two storage experiments (Tables XXV and XXVI). A reversal of trends during storage was observed in both tests.

4.5.- Viscosity at 50° C. This parameter changed in a similar way to peak viscosity (Tables XXV and XXVI). Sample II.5 (Table XXV), highly deteriorated by the end of the experiment, showed a significant decrease in viscosity at 50° C.

4.6.- Setback. During the first five months of the 1964-65 test, setback values were positive and underwent significant changes. Undermilled rices showed a gradual increase. White milled rices showed a first decrease and then increased. With increasing storage time slight decreases have been found. The decrease exhibited by the undermilled rice sample stored in an aerated cabinet at $\pm 25^{\circ}$ C was remarkable: near 250 B.U. (Table XXV Fig. 16).

The 1965-66 test results (Table XXVI) confirmed previous data. In undermilled rices, setback increased during the first months, this change being positively associated with temperature. In certain samples, a reversal of trends was initiated (changing to a definite decrease) as it was found in the 1964-65 tests. Though data for well milled rice were not completed, it seems that the now available information is not opposite to that obtained in the previous test. For instance, setback values of sample IV-C-3 (well milled rice, air-tight

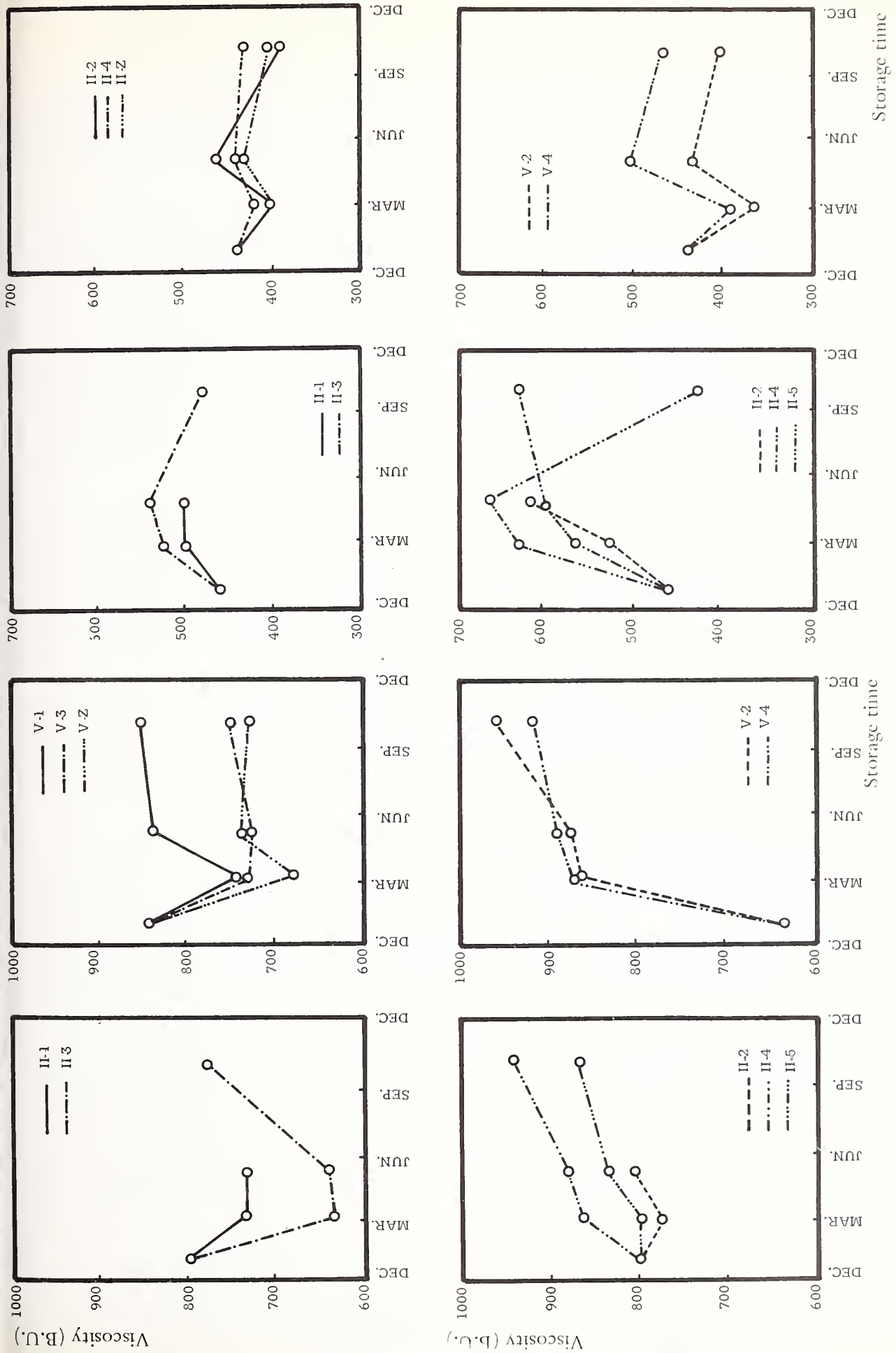


Fig. 15.- Changes during storage in the peak viscosity of amilograms of undermilled and white milled rice (1964 - 65 experiment).

Fig. 16.- Changes during storage in the set back value of undermilled and white milled rice (1964 - 65 experiment)

stored at $+35^{\circ}\text{C}$ with 15.5% M.C.) exhibited a quick decrease after the third month, it being parallel to deterioration. This is in good agreement with the trends observed at the end of the 1964-65 storage experiment.

Changes in amylograms (Table XXV) were not correlated with those in organoleptic properties of the cooked samples (Fig. 12). In general, a parallel increase of peak viscosity, set-back and quality could be admitted but this relationship did not hold a detailed comparison.

Results obtained indicate that the amylogram is not a reliable and useful means to follow and interpret changes taking place in milled rice during storage. The lack of constant and defined changes in its characteristic points limits its practical use. It appears that more should be learnt about the fate of lipids, proteins, enzymes, microflora, etc. and their interactions during aging, before the pasting characteristics of rice, as determined by the amylograms, can be better understood.

5. N INDEX

Fig. 17 shows the full sequence of changes during the ten months of the 1966-67 storage experiment. Data show the occurrence of important changes in the chemical composition -protein material- of the outer layer.

The trends of changes are closely related to holding conditions, particularly to temperature: At $+5^{\circ}\text{C}$, N index remains constant during a first period but after 2-4 months increases. At $+25^{\circ}\text{C}$, N index increases slowly; however prolonged storage brings about a decrease when deterioration of rice appears. At $+35^{\circ}\text{C}$, N index decreases -as soon as rice deteriorates.

Each type of change (increase and decrease) seems to have a different cause, being in mind that N index is an empirical measurement related to alkali soluble protein in the outer layer. The increase is supposed to be due to breakdown of protein complexes -i.e. lipoproteins, or glycoproteins- via enzymatic actions. The decrease might be brought about by an interaction of carbonyl compounds -from lipid deterioration- with proteins. Experimental data supporting the first explanation are not available. However, some evidence supporting the view of the carbonyl-protein interaction is presented in Part III of present report.

Possibility of occurrence of this interaction seems worthy to be studied; it might also have consequences on characteristics other than the eating quality of rice. Carbonyl groups would preferentially react with amino groups such as those in lysine, affecting directly the nutritive value of rice.

Relationship between N index changes and quality variation is commented elsewhere (see Part III).

6. SH AND SS INDICES

In previous investigations (see Part III of present report) a tentative method for measuring the quality of rice was developed based on the relationship found between

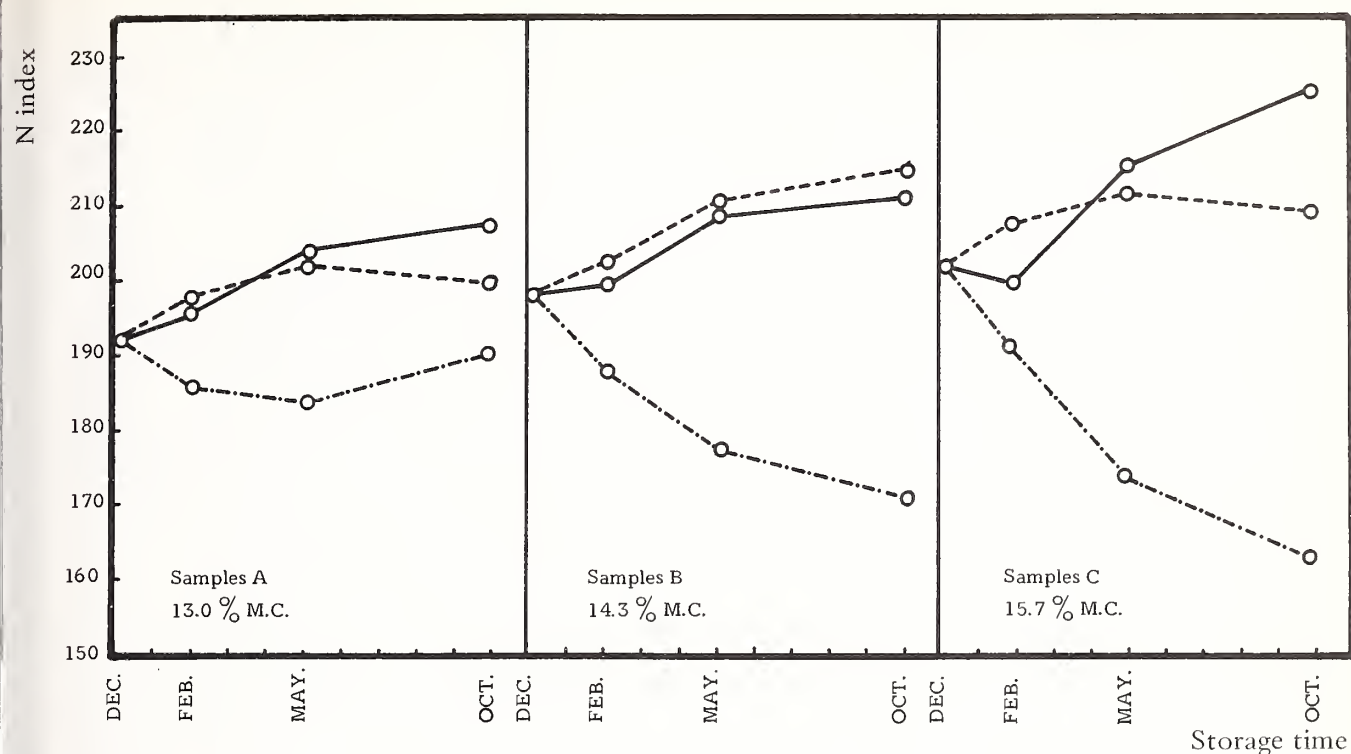


Fig. 17.- Changes in the N index of rice during storage (1966 - 67 experiment).

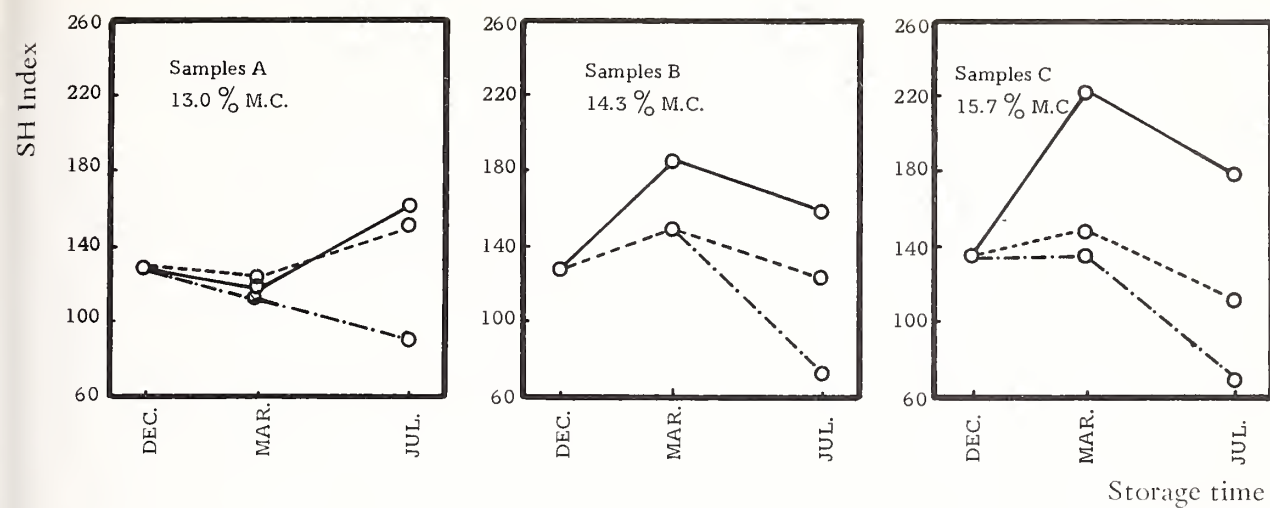


quality and the SS groups content of milled rice. The procedure has been applied to follow changes during storage of milled rice, in order to obtain further information on the possible relationships among SH and SS indices and the properties of rice.

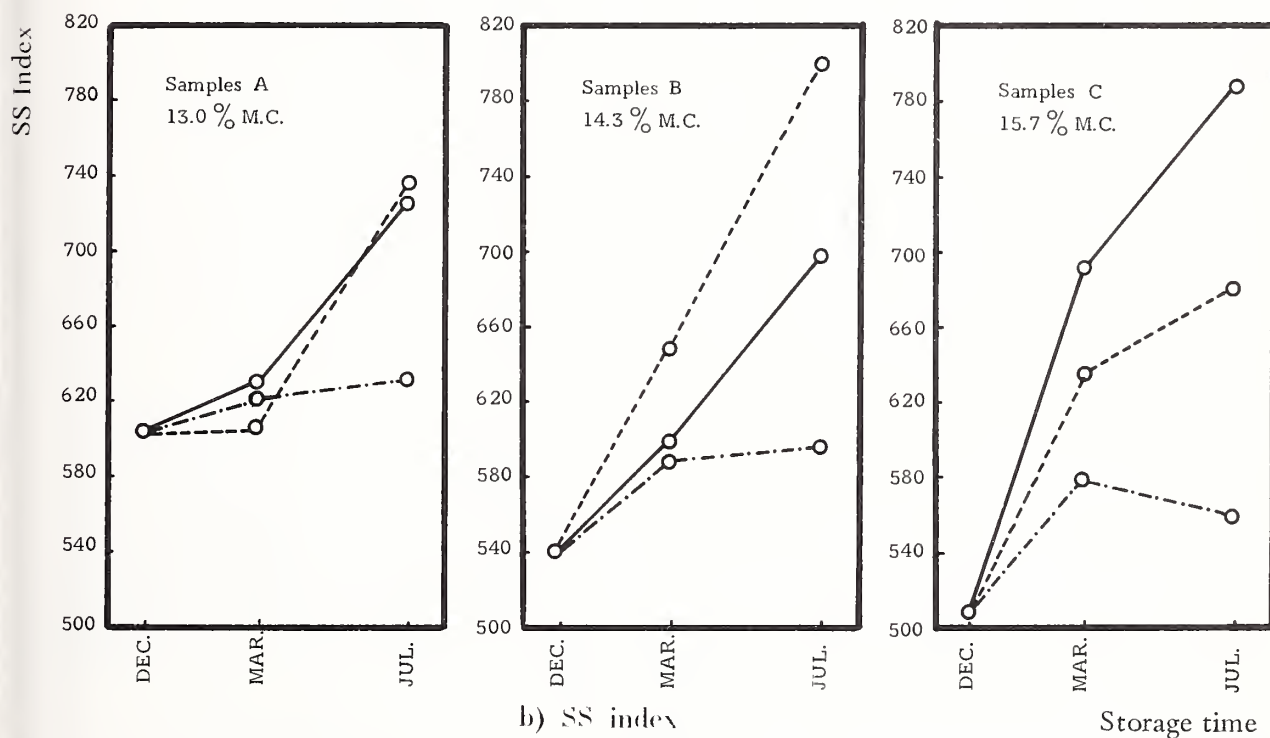
Fig. 18 shows changes found in SH and SS indices during storage of milled rice samples held at various moisture and temperature levels. Storage results in important changes in both parameters. SH as well SS groups are highly labile and undergo changes readily even at +5°C. In general, an increase takes place, followed by a decrease well after deterioration of rice. This reversal of the trends of changes does not occur simultaneously in both parameters. SH reaches the peak level first. The significance of these changes is unknown. As it will be dealt with further on (Part III) they are not related to changes observed in the properties of rice.

7. INTERGRANULAR AIR

Formation of carbon dioxide in air-tight stored rice was detected in 1964-65 and 1965-66 experiments. The purposes of 1966-67 work were: a) to measure the variation of



a) SH index



b) SS index

Fig. 18.- Changes in the SH and SS indices of rice during storage (1966-67 experiment)

— at + 5°C
 - - - at + 25°C
 - · - · at + 35°C

Table XXIX.- Effects of storage on CO₂ concentration in intergranular air and germinative capacity of milled rice.

Samples (1)	Storage time	% CO ₂							
		Dec. 13	Jan. 23	Feb. 14	Mar. 7	May 8	May 29	Jul. 19	Sept. 18
A - 1		0.06	0.06	0.06	0.08	0.08	0.08	0.1	0.09
A - 2		0.06	0.2	0.2	0.2	0.3	0.4	0.7	0.7
A - 3		0.06	0.9	1.1	1.2	2.0	2.8	2.5	2.5
B - 1		-	0.09	0.1	0.1	0.1	0.09	0.1	0.1
B - 2		-	0.3	0.4	0.6	2.4	7.2	8.2	5.3
B - 3		-	10.6	12.2	10.3	13.3	13.3	11.9	6.9
C - 1		-	0.2	0.2	0.2	0.3	0.3	0.6	0.6
C - 2		-	8.3	8.9	9.4	11.2	12.3	12.5	10.4
C - 3		-	13.2	16.9	15.7	16.6	17.8	14.3	9.9

	Dec. 13	Jan. 23	Feb. 14	Mar. 7	May 8	May 29	Jul. 19	Sept. 18
A - 1	-	96	90	94	81	79	-	73
A - 2	-	95	78	78	64	-	-	0
A - 3	-	0	0	0	0	-	-	-
B - 1	-	97	82	82	82	79	-	75
B - 2	-	89	29	13	0	0	-	-
B - 3	-	0	0	0	0	-	-	-
C - 1	-	92	59	73	69	31	-	28
C - 2	-	36	1.5	0	0	-	-	-
C - 3	-	0	0	0	0	-	-	-

(1) See Table XX for identification.

actual concentrations of CO₂ in intergranular air as a function of storage time and holding conditions, and b) to compare CO₂ evolution, germinative capacity and microflora changes, in order to obtain information on the participation of the latter factors in the formation of CO₂.

The problem was approached in two ways: 1st) Quantitative GLC analysis of CO₂ in intergranular atmosphere, tetrazolium test for germinative capacity, and microbiological analysis of bacteria, molds and yeast counts were carried out frequently during storage in all samples of the 1966-67 storage experiment. 2nd) Germed and hand-degermed samples of undermilled rice were stored in air-tight bottles under accelerated storage conditions (14.8% moisture content, 35°C); CO₂, germinative capacity (tetrazolium test) and microflora (per cent of kernels from which viable mold colonies can be obtained) were determined at frequent intervals during 26 days.

Table XXIX reports results of CO₂ and germinative capacity for the nine rice samples of the 1966-67 storage experiment. Evolution of CO₂ depends on moisture content of rice and

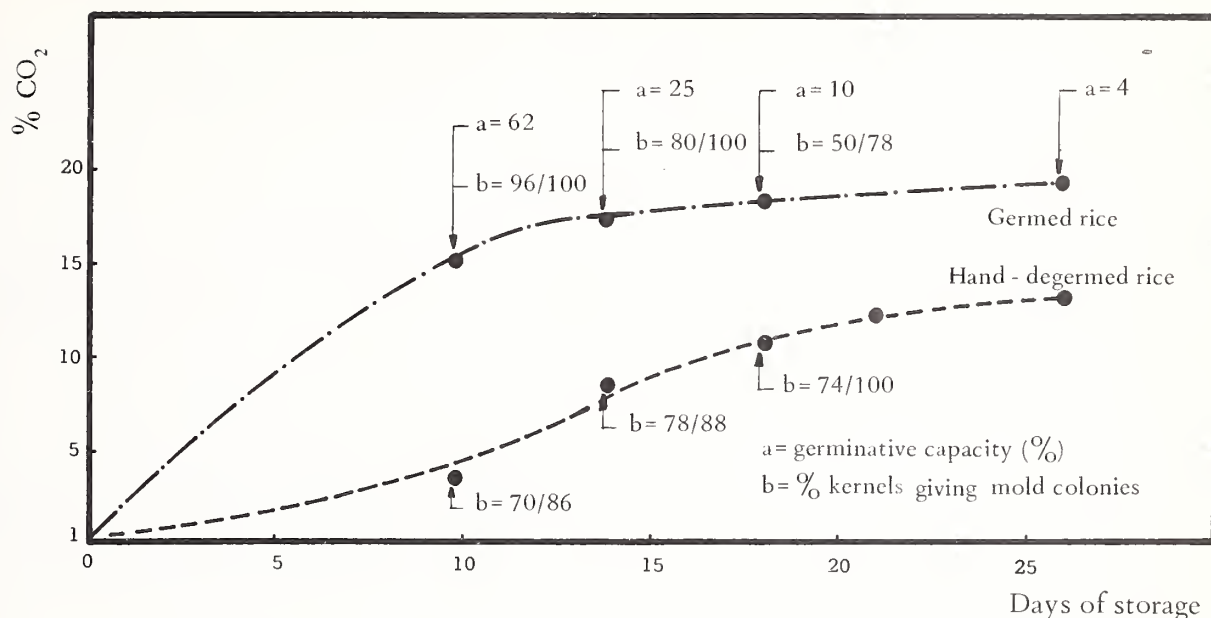


Fig. 19.- Changes in CO₂ in intergranular air, germinative capacity and % kernels giving mold colonies, during air-tight storage of milled rice (M.C. = 14.8 %; storage temperature = 35°C).

holding temperature. At +5°C, changes in CO₂ are negligible; CO₂ proportion amounts to 0.5% only in samples with 15.7% M.C. stored during ten months. At +25°C, CO₂ evolution is greatly dependent on moisture content: 13.0% M.C., 0.7% CO₂ in ten months; 14.8% M.C., 0.7% CO₂ in about three months; and 15.7% M.C., 8.3% CO₂ in only one month. At +35°C changes of similar trends but at a faster rate occur. It should be added that under comparable storage conditions, undermilled rices generated CO₂ at a faster rate than well milled rices.

It will be noted that the proportion of CO₂ in intergranular air declines at the completion of the storage period. This fact has also been observed in previous experiments, although no explanation has been found.

Results of tetrazolium test are included in Table XXIX. The rate of CO₂ evolution decreases sharply once the germinative capacity is lost. However some further increase in CO₂ concentration takes place. Other causes than germinative capacity must therefore contribute to CO₂ formation. Microbiological data do not help to explain them.

Results from the accelerated storage test with germed and hand-degermed rice samples (Fig. 19) supplied more clear information. They showed that rice germ, with germinative capacity, contributes substantially to CO₂ in intergranular air. Rice having germ developed CO₂ earlier and at a faster rate than rice without germ. After ten days of storage, intergranular air of germed rice samples contained 16% CO₂ whereas that of hand-degermed rice contained 3%. It is important to point out that both types of samples had similar percentages of infected kernels (kernels giving mold colonies).

It must be mentioned however that CO_2 levels in intergranular atmosphere of degermed rice also were remarkable. Therefore, participation of microbial respiration in CO_2 production appears to be important.

III. EFFECTS OF STORAGE ON THE CHEMICAL COMPOSITION OF MILLED RICE

1. CARBOHYDRATES

1.1. Starch.

Starch content remained practically unchanged during storage of milled rice (Table XXX). Small decreases were noticed in some of the undermilled samples. The high microbial contamination of these samples (bacteria: 287,000 per gram; molds and yeasts: 133,000 per gram) might perhaps account for the small starch losses. Gosh (269) has shown that fungal deterioration results in significant starch losses.

1.2. Amylose.

Amylose content of rice did not undergo significant changes (Table XXX); although it increased slightly during the first period of storage, no further change was detected with increasing storage time. It was not investigated whether the small changes found during storage were due to an actual variation of amylose content or to the increase (known to take place (250) (251)) in the iodine combining capacity of the linear starch component.

1.3. Sugars.

1.3.a. Changes in the entire kernel. Values for total (TS), reducing (RS) and non reducing (NRS) sugars contents of milled rices from three storage experiments are given in Tables XXXI and XXXII. Data show that RS content increased and NRS content decreased during storage of milled rice. In general, changes were not important; with the only exception of those registered in samples under the most severe conditions, changes were slow and small, they being difficult to be detected within the first months of storage. It can be observed that changes in RS and NRS were not compensated; the latter were larger and, therefore, TS decreased. The trends of changes were similar to those previously reported for storage of rough and brown rice (see Literature Review, Part II).

Although changes were small, reported data indicated that milling degree, moisture content and temperature are influencing factors. As it was expected, the lower the milling degree (less bran removed), and the higher the moisture content and temperature, the larger the changes are.

1.3.b. Changes in outer layer as compared to those in nucleus and entire kernel.

Data from two storage experiments are given in Tables XXXIII and XXXIV. In the outer layer, RS increased and NRS as well as TS decreased. The pattern was similar to that of in the entire

Table XXX.- Changes in carbohydrate contents of milled rice during storage. I. Starch and amylose (1964-65 experiment).

Samples (1)	Storage time (2)	Starch %			
		0	1	2	3
II - 1		83.56	83.20	80.92	-
II - 2		83.56	83.48	82.77	-
II - 3		83.56	84.10	80.27	81.71
II - 4		83.56	84.53	82.29	83.06
II - 5		83.56	84.68	81.54	82.93
V - 1		87.36	87.16	86.85	85.40
V - 2		87.36	86.58	87.40	87.17
V - 3		87.36	86.92	87.11	-
V - 4		87.36	85.74	86.92	86.99
V - z		87.36	86.97	86.67	-
		Amylose %			
		0	1	2	3
II - 1		8.97	-	11.10	-
II - 2		8.97	-	10.40	-
II - 3		8.97	-	11.80	10.46
II - 4		8.97	-	9.90	9.83
II - 5		8.97	-	10.45	10.07
V - 1		9.79	-	11.40	11.46
V - 2		9.79	-	11.70	11.52
V - 3		9.79	-	11.45	10.74
V - 4		9.79	-	12.02	11.56
V - z		9.79	-	11.30	10.84

(1) See Table XVIII for identification.

(2) Starch determinations were carried out at the beginning of storage (time 0), and after three (time 1), five (time 2) and ten months (time 3). Amylose determinations were carried out at the beginning of storage (time 0), and after five (time 2) and eleven months (time 3).

Table XXXI.- Changes in carbohydrate content of milled rice during storage. II. Sugars (1965-66 experiment).

Samples (3)	Storage time(4)	Sugars								
		Reducing sugars(1)			Non reducing sugars(2)			Total sugars (%)		
		0	1	2	0	1	2	0	1	2
II-A.4		0.14	-	0.16	0.51	-	0.52	0.65	-	0.69
II-A.1		0.14	-	0.18	0.51	-	0.48	0.65	-	0.66
II-A.2		0.14	0.13	0.19	0.51	0.53	0.49	0.65	0.66	0.68
II-A.3		0.14	0.17	0.22	0.51	0.32	0.15	0.65	0.49	0.37
II-B.4		0.14	-	-	0.47	-	-	0.61	-	-
II-B.1		0.14	-	0.15	0.47	-	0.53	0.61	-	0.69
II-B.2		0.14	0.15	0.17	0.47	0.38	0.35	0.61	0.53	0.53
II-B.3		0.14	0.31	0.36	0.47	0.07	0.04	0.61	0.38	0.40
II-C.4		0.15	0.15	0.16	0.50	0.49	0.50	0.65	0.64	0.67
II-C.1		0.15	0.17	0.19	0.50	0.48	0.49	0.65	0.65	0.68
II-C.2		0.15	0.36	0.42	0.50	0.19	0.14	0.65	0.55	0.56
II-C.3		0.15	0.45	0.47	0.50	0.09	0.05	0.65	0.54	0.52
IV-A.4		0.09	-	-	0.20	-	-	0.29	-	-
IV-A.1		0.09	-	-	0.20	-	-	0.29	-	-
IV-A.2		0.09	0.09	-	0.20	0.15	-	0.29	0.24	-
IV-A.3		0.09	0.13	0.15	0.20	0.05	0.04	0.29	0.18	0.19
IV-B.4		0.08	-	-	0.17	-	-	0.25	-	-
IV-B.1		0.08	-	-	0.17	-	-	0.25	-	-
IV-B.2		0.08	0.13	-	0.17	0.13	-	0.25	0.26	-
IV-B.3		0.08	0.18	-	0.17	0.03	-	0.25	0.21	-
IV-C.4		0.08	-	0.10	0.17	-	0.11	0.25	-	0.21
IV-C.1		0.08	0.11	0.11	0.17	0.14	0.11	0.25	0.25	0.22
IV-C.2		0.08	0.17	0.20	0.17	0.04	0.03	0.25	0.21	0.23
IV-C.3		0.08	0.19	0.17	0.17	0.03	0.01	0.25	0.22	0.18
II-X.2		0.14	0.10	-	0.47	0.14	-	0.61	0.24	-
IV-X.2		0.08	-	-	0.17	-	-	0.25	-	-

(1) g maltose/100 g rice.

(2) g sucrose/100 g rice.

(3) See Table XIX for identification.

(4) Determinations were carried out at the beginning of storage (time 0), and after three (time 1) and five months (time 2).

Table XXXII.- Changes in carbohydrate content of milled rice during storage. II. Sugars (1964-65 and 1966-67 experiments).

Samples (1)	Storage time(4)	Reducing ⁽²⁾ %			Non reducing ⁽³⁾ %			Total %		
		0	1	2	0	1	2	0	1	2
II - 1		0.14	0.15	-	0.71	0.66	-	0.85	0.81	-
II - 2		0.14	0.13	-	0.71	0.62	-	0.85	0.75	-
II - 3		0.14	0.12	0.16	0.71	0.61	0.57	0.85	0.73	0.73
II - 4		0.14	0.13	0.20	0.71	0.60	0.32	0.85	0.73	0.52
II - 5		0.14	0.13	0.24	0.71	0.55	0.11	0.85	0.68	0.35
V - 1		0.09	0.11	0.10	0.20	0.18	0.20	0.29	0.29	0.30
V - 2		0.09	0.09	0.10	0.20	0.18	0.18	0.29	0.27	0.28
V - 3		0.09	0.09	0.10	0.20	0.21	0.20	0.29	0.30	0.30
V - 4		0.09	0.08	0.10	0.20	0.17	0.15	0.29	0.25	0.25
V - z		0.09	0.10	0.11	0.20	0.20	0.18	0.29	0.30	0.29
A - 1		0.12	0.13	-	0.26	0.27	-	0.38	0.39	-
A - 2		0.12	0.13	-	0.26	0.30	-	0.38	0.42	-
A - 3		0.12	0.13	-	0.26	0.19	-	0.38	0.31	-
B - 1		0.12	0.15	-	0.26	0.24	-	0.38	0.38	-
B - 2		0.12	0.16	-	0.26	0.25	-	0.38	0.41	-
B - 3		0.12	0.22	-	0.26	0.08	-	0.38	0.29	-
C - 1		0.12	0.16	-	0.26	0.25	-	0.38	0.40	-
C - 2		0.12	0.18	-	0.26	0.20	-	0.38	0.37	-
C - 3		0.12	0.31	-	0.26	0.03	-	0.38	0.33	-

(1) See Tables XVIII and XX for identification. (2) g maltose/100 g rice, d.b. (3) g sucrose/100 g rice, d.b. (4) Determinations were carried out in December (time 0), March (time 1) and October (time 2) in 1964-65 experiment and in January (time 0) and April (time 1) in 1966-67 experiment.

kernel. The magnitude of sugar changes was several times greater in the outer layer than in the nucleus. In the outer layer, NRS losses were greater than RS increases whereas the reverse occurred in the nucleus. Consequently, TS content of outer layer increased and that of nucleus decreased. The influence of outer layer predominated in the entire kernel, although changes here were much more small due to participation of nucleus.

That storage changes in sugars of rice are influenced by milling degree, moisture content and temperature is more clearly shown by outer layer data than by results for the entire kernel. A major part of total changes in the kernel occurs in the outer layer. Consequently, data of this outer portion of the kernel supply a more actual information on the effects of storage on rice than those of the average composition of the entire kernel. Moreover, changes in this layer can be detected earlier.

Table XXXIII.- Comparison of changes in reducing, non reducing and total sugar contents of outer layer, nucleus and entire kernel of milled rice during storage (1965-66 experiment).

Samples (1)	Storage time(2)	Entire kernel		Outer layer ⁽⁵⁾		Nucleus	
		0	1	0	1	0	1
Reducing sugars ⁽³⁾							
II-C.4		0.15	0.16	0.50	0.64	0.08	0.12
II-C.1		0.15	0.19	0.50	0.90	0.08	0.13
II-C.2		0.15	0.42	0.50	1.54	0.08	0.24
II-C.3		0.15	0.47	0.50	1.05	0.08	0.35
II-B.3		0.14	0.36	0.50	1.35	0.08	0.15
II-A.3		0.14	0.22	0.50	0.94	0.08	0.12
IV-C.4		0.08	0.10	0.37	0.35	0.07	0.10
IV-C.1		0.08	0.11	0.37	0.36	0.07	0.14
IV-C.2		0.08	0.20	0.37	0.69	0.07	0.13
IV-C.3		0.08	0.17	0.37	0.47	0.07	0.14
Non reducing sugars ⁽⁴⁾							
II-C.4		0.50	0.50	3.52	3.37	0.09	0.08
II-C.1		0.50	0.49	3.52	3.26	0.09	0.06
II-C.2		0.50	0.14	3.52	0.51	0.09	0.04
II-C.3		0.50	0.05	3.52	0.32	0.09	0.02
II-B.3		0.47	0.04	3.52	0.12	0.09	0.02
II-A.3		0.51	0.15	3.52	1.04	0.09	0.05
IV-C.4		0.17	0.11	0.86	0.66	0.05	0.03
IV-C.1		0.17	0.11	0.86	0.65	0.05	0.02
IV-C.2		0.17	0.03	0.86	0.12	0.05	0.04
IV-C.3		0.17	0.01	0.86	0.02	0.05	0.01
Total sugars %							
II-C.4		0.65	0.67	4.02	4.01	0.17	0.20
II-C.1		0.65	0.68	4.02	4.16	0.17	0.19
II-C.2		0.65	0.56	4.02	2.05	0.17	0.28
II-C.3		0.65	0.52	4.02	1.37	0.17	0.37
II-B.3		0.61	0.40	4.02	1.47	0.17	0.17
II-A.3		0.65	0.37	4.02	1.98	0.17	0.17
IV-C.4		0.25	0.21	1.23	1.01	0.13	0.13
IV-C.1		0.25	0.22	1.23	1.01	0.13	0.16
IV-C.2		0.25	0.23	1.23	0.81	0.13	0.17
IV-C.3		0.25	0.18	1.23	0.49	0.13	0.15

(1) See Table XIX for identification. (2) Determinations were carried out at the beginning of the storage (time 0) and after five months (time 1). (3) g maltose/100 g rice. (4) g sucrose/100 g rice. (5) 10% of the kernel weight.

Table XXXIV.- Comparison of changes in reducing, non reducing and total sugar contents of outer layer, nucleus and entire kernel of milled rice during storage (1966-67 experiment).

Samples (1)	Storage time(2)	Reducing sugars (3)					
		Entire kernel		Outer layer		Nucleus	
		0	1	0	1	0	1
B - 1		0.12	0.15	0.50	0.52	0.07	0.11
B - 2		0.12	0.16	0.50	0.63	0.07	0.12
B - 3		0.12	0.22	0.50	0.98	0.07	0.17
A - 2		0.12	0.13	0.50	0.52	0.07	0.08
		Non reducing sugars (4)					
		0	1	0	1	0	1
B - 1		0.26	0.24	2.42	1.94	0.11	0.09
B - 2		0.26	0.25	2.42	1.96	0.11	0.11
B - 3		0.26	0.08	2.42	0.38	0.11	0.05
A - 2		0.26	0.30	2.42	1.97	0.11	0.10
		Total sugars %					
		0	1	0	1	0	1
B - 1		0.37	0.38	2.88	2.44	0.18	0.20
B - 2		0.37	0.41	2.88	2.55	0.18	0.21
B - 3		0.37	0.29	2.88	1.27	0.18	0.21
A - 2		0.37	0.42	2.88	2.44	0.18	0.18

(1) See Table XX for identification of samples. (2) Determinations were carried out in January (time 0) and April (time 1). (3) g maltose/100 g rice, d.b. (4) g sucrose/100 g rice, d.b.

2. NITROGEN COMPOUNDS

2.1. Total (TN), non protein (NPN) and protein nitrogen (PN).

2.1.a. Changes in the entire kernel. TN, NPN and PN remained practically unchanged during storage of milled rice (Table XXXV), even in samples held under adverse conditions. NPN values showed a tendency to decrease with storage time, however, NPN concentration levels were very low (0.01 - 0.03%) and changes negligible.

TN has been reported to remain constant in rices stored under conditions different from those studied in present experiment (92). The fact that this is a normal behavior of cereals stored under ordinary conditions (270), can be extended, on the basis of the now obtained data, to hermetic storage of rice.

2.1.b. Changes in outer layer as compared to those in nucleus and entire kernel. There were no significant changes in TN, PN and NPN contents of outer layer nor in those of nucleus (Table XXXVI), it confirming the results found for the entire kernel. The lack of changes in TN, PN and NPN permits to extend to old milled rice the assumption that TN reflects PN (see pg.44).

Table XXXV.- Changes in nitrogen compounds of milled rice during storage. I. Total (TN), protein (PN) and non protein nitrogen (NPN) (1964-65 and 1966-67 experiments).

Samples (1)	Storage time(2)	Total nitrogen %				Protein nitrogen %				Non protein nitrogen %			
		0	1	2	3	0	1	2	3	0	1	2	3
II - 1		1.63	1.64	1.65	-	1.60	1.61	1.63	-	0.03	0.03	0.02	-
II - 2		1.63	1.59	1.61	-	1.60	1.57	1.59	-	0.03	0.02	0.02	-
II - 3		1.63	1.59	1.59	1.55	1.60	-	1.58	1.54	0.03	-	0.01	0.01
II - 4		1.63	1.59	1.60	1.56	1.60	1.56	1.58	1.55	0.03	0.03	0.02	0.01
V - 1		1.58	-	1.61	1.55	1.57	-	1.59	-	0.01	-	0.02	-
V - 2		1.58	1.57	1.53	1.55	1.57	1.56	1.53	1.54	0.01	0.01	0.01	0.01
V - z		1.58	1.59	1.61	1.55	1.57	1.58	1.60	1.54	0.01	0.01	0.01	0.01
V - 3		1.58	-	1.58	1.46	1.57	-	1.57	1.45	0.01	-	0.01	0.01
V - 4		1.58	-	1.56	1.48	1.57	-	1.55	-	0.01	-	0.01	-
B - 1		1.39	-	1.33	-	1.37	-	1.32	-	0.02	-	0.01	-
B - 2		1.39	-	1.31	-	1.37	-	1.30	-	0.02	-	0.01	-
B - 3		1.39	-	1.29	-	1.37	-	1.28	-	0.02	-	0.01	-

(1) See Tables XVIII and XX for identification. (2) Determinations were carried out at the beginning of the storage (time 0) and after three (time 1), five (time 2) and ten months (time 3).

Table XXXVI.- Comparison of changes in total (TN), protein (PN) and non protein nitrogen (NPN) contents of outer layer, nucleus and entire kernel of milled rice during storage (1966-67 experiment).

Samples (1)	Storage time(2)	Entire kernel		Outer layer (3)		Nucleus	
		0	1	0	1	0	1
Total nitrogen %							
B - 1		1.39	1.33	2.53	2.53	1.27	1.27
B - 2		1.39	1.31	2.53	2.48	1.27	1.22
B - 3		1.39	1.29	2.53	2.53	1.27	1.20
Non protein nitrogen %							
B - 1		0.02	0.01	0.05	0.05	0.02	0.01
B - 2		0.02	0.01	0.05	0.05	0.02	0.01
B - 3		0.02	0.01	0.05	0.04	0.02	0.01
Protein nitrogen %							
B - 1		1.37	1.32	2.49	2.48	1.25	1.26
B - 2		1.37	1.30	2.49	2.43	1.25	1.21
B - 3		1.37	1.28	2.49	2.50	1.25	1.19

(1) See Table XX for identification.

(2) Determinations were carried out in December-January (time 0) and in May-June (time 1).

(3) 5% of kernel weight.

2.2. Protein solubility fractions.

2.2.a. Changes in the entire kernel. Protein extraction yields decreased during storage (Table XXXVII). The extent of loss varied with storage conditions; the more unsafe the conditions the larger the loss was. This amounted to 25% in sample with 14.3% M.C., held at +35°C six months. As it is shown in the table, every protein solubility fraction contributed to the loss in total protein extraction yield. Decreases in various protein solubility fractions of rice during storage have been reported by different authors (92)(205)(274)(301). In hulled rice, it has been found that all protein fractions other than albumins, are appreciably decomposed during storage (312).

Glutelins accounted for most of the total losses. However, as a percentage of the original fraction, albumins and prolamins underwent the highest decrease. In sample B-3 (14.3% M.C., +35°C) the loss in latter fractions were 40% and 48% respectively, whereas the loss in globulins and glutelins were 32% respectively.

2.2.b. Changes in outer layer as compared to those in nucleus and entire kernel. Losses in protein extraction yields were somewhat higher at the outer than at the inner region of the kernel (Table XXXVII). As a result, extraction yield in outer layer was significantly lower than in nucleus.

Glutelins accounted for a major part of total absolute losses in outer layer, as it was the case in the entire kernel. Likewise, albumins and prolamins underwent the higher decrease as a percentage of the original fraction.

The percent loss in each protein solubility fraction was not the same in the outer and in the inner region of the kernel. Albumins and prolamins losses in the outer layer were larger than in the nucleus (56% and 70%, and 41% and 50% respectively in sample B-3. Table XXXVII). Inversely, globulins and glutelins losses were larger in the nucleus.

The ratio of albumins: globulins; prolamins: glutelins in the outer layer was affected somewhat by storage. In sample B-3, it changed from 15:10:6:69 to 10:11:3:76. However, it did not change in the nucleus (5:10:4:81 before storage and 4:10:3:83 after storage).

Examination of glutelin changes shows that after 4-5 months of storage, this fraction increases somewhat in the outer layer of the sample held at +5°C. The change is rather small and should therefore be considered with reserve. Nevertheless, it must be pointed out that the amount of proteins extracted from the entire kernel by the N index procedure, has also been found to increase during storage of milled samples held at moderate and low temperatures (see Fig. 17). Whether the increase of alkali soluble protein -under certain storage conditions and time- is due or not to breakdown of glyco- and/or lipoproteins, catalysed by enzymatic activities concentrated in the outer layer, has not been investigated yet.

2.3. Sulphydryl and disulfide contents.

The relationship found in a previous work between SS groups and the quality of rice (see Part III) prompted us to investigate storage changes in SH and SS contents of rice. Preliminary data (obtained in 1964-65) showed that storage results in SH and SS changes, the trends

Table XXXVII.- Effects of storage on protein solubility fractions of milled rice: Comparison of changes in outer layer, nucleus and entire kernel.

	Rice samples ⁽¹⁾						
	1966-67 experiment				1967-68 experiment		
	B	+5°C	+25°C	+35°C	+5°C	+25°C	+35°C
Albumins(2)							
Entire kernel	0.30	0.38	0.25	0.18	0.35	-	0.21
Outer layers(3)	1.75	1.44	1.44	0.79	0.64	0.49	0.56
Nucleus	0.29	0.27	0.16	0.17	-	-	-
Globulins(2)							
Entire kernel	0.67	0.57	0.59	0.45	0.19	-	0.17
Outer layer(3)	1.12	0.71	0.65	0.89	0.76	0.60	0.65
Nucleus	0.60	0.45	0.63	0.44	-	-	-
Prolamins(2)							
Entire kernel	0.25	0.14	0.08	0.13	0.15	-	0.16
Outer layer(3)	0.72	0.19	0.19	0.21	0.29	0.22	0.20
Nucleus	0.22	0.10	0.10	0.11	-	-	-
Glutelins(2)							
Entire kernel	5.25	4.90	4.81	3.74	6.48	-	6.09
Outer layer(3)	7.93	8.85	7.84	6.00	11.71	9.34	9.13
Nucleus	5.05	4.10	4.36	3.41	-	-	-
Total soluble(2)(4)							
Entire kernel	6.47	5.99	5.73	4.50	7.17	-	6.63
Outer layer(3)	11.62	11.19	10.12	7.89	13.40	10.65	10.54
Nucleus	6.16	4.92	5.25	4.13	-	-	-
Insoluble(2)							
Entire kernel	1.68	1.87	1.98	3.11	2.41	-	2.95
Outer layer(3)	3.17	3.58	4.33	6.96	4.78	6.60	7.91
Nucleus	1.27	2.58	1.96	2.93	-	-	-
% Extraction yield							
Entire kernel	79.3	76.1	74.3	59.0	74.8	-	69.2
Outer layer(3)	77.9	75.7	70.0	53.1	73.7	62.3	57.1
Nucleus	80.7	71.9	72.7	58.5	-	-	-

(1) See Table XX and XXI for identification.

(2) g/100 g rice, dry basis.

(3) 5% of the kernel weight.

(4) Sum of extracted fractions.

of which seemed to be alternating with storage time. Later studies on the distribution and role of these functional groups in rice (see Parts I and III), indicated that SH and SS levels in outer layer are higher than in the entire kernel and more operative in the rice behaviour. The 1965-66 storage experiment showed that only the changes in outer layer were large enough to show clear trends: both SH and SS contents decreased. Some minor exceptions in samples held at low temperatures, -20° or $\pm 5^{\circ}\text{C}$, were noted. Results from a subsequent storage experiment (1966-67) (Table XXXVIII) confirmed the final loss of both SH and SS groups with storage time, in agreement with the changes found in SH and SS indices after prolonged storage (see Fig. 18). Once more the sample held at $\pm 5^{\circ}\text{C}$, with lower moisture content, was an exception. The decrease of SS contents indicated the occurrence of changes in the chemical characteristics of the protein material concomitant with the loss of solubility commented above.

Although there is a lack of information on SH and SS changes in rice, those regarding wheat show that SH content decrease (313); moisture (313), metallic catalysts (314) and oxidized flour lipids (314)(315) facilitate SH oxidation. Our results are not in disagreement with this information.

It has been reported that the decrease in SH groups may be due to the oxidation to a disulfide bond or to a sulfoxide (313). Our results have not shown increase in SS bonds. Similarly, Tsen and Bushuk (316) have reported losses both in SH and SS contents of dough mixing under various conditions. In a related paper Sullivan and Dahle (317) have reported that "although, theoretically, SS-SH interchange had seemed a logical explanation for the changes in the rheological properties of flour doughs effected by improvers, results have shown this reaction may not, in fact, take place to any significant degree in the normal pH".

2.4. Free amino nitrogen.

2.4.a. Changes in the entire kernel. Free amino N (FAN) content of the entire kernel underwent non significant changes during storage of milled rice (Table XXXIX). These results were in contrast with those reported previously for brown rice (274)(84)(280) and parboiled rice (137) stored in non hermetic containers, and according to which, free amino N decreases. It appears that FAN changes occur much more rapidly in brown than in milled rice, although factors other than milling degree (such as intergranular atmosphere and micro-flora) may also have a definite influence on FAN changes in stored rice.

2.4.b. Changes in outer layer as compared to those in nucleus and entire kernel. Data from two storage experiments are given in Table XL. Unlike in the entire kernel, FAN changes in the outer layer were significant. Clear losses were observed in most samples, they being in agreement with the general pattern commented above. On the contrary, FAN content of nucleus remained practically unchanged. This and the high weight proportion of nucleus as compared with that of the outer layer, explains why changes in the entire kernel are negligible in spite of the significant losses that occur in outer layer. Separate consideration of outer layer affords a more actual and sensitive means to follow storage changes than the entire kernel.

Table XXXVIII.-Effects of storage on SH and SS contents of milled rice: Comparison on changes in outer layer, nucleus and entire kernel (1966-67 experiment).

Samples (2)	Storage time(3)	Sulphydryl groups (4)						
		Entire kernel		Outer layer (1)			Nucleus	
		0	1	0	1	2	0	1
A - 1		1.27	0.98	3.30	2.75	2.19	1.17	0.93
A - 2		1.27	1.24	3.30	3.02	2.88	1.17	1.16
A - 3		1.27	1.07	3.30	2.93	2.52	1.17	0.96
B - 1		1.27	1.01	3.30	2.91	2.82	1.17	0.95
B - 2		1.27	1.21	3.30	2.89	2.66	1.17	1.13
B - 3		1.27	1.25	3.30	2.51	2.31	1.17	1.18
C - 1		1.27	1.23	3.30	-	-	1.17	-
C - 2		1.27	1.13	3.30	-	-	1.17	-
C - 3		1.27	1.15	3.30	-	-	1.17	-
		Disulfide groups (4)						
		Entire kernel		Outer layer (1)			Nucleus	
		0	1	0	1	2	0	1
A - 1		4.50	4.52	12.85	15.00	8.58	4.02	3.97
A - 2		4.50	4.33	12.85	11.40	8.08	4.02	3.68
A - 3		4.50	3.75	12.85	12.00	8.91	4.02	3.56
B - 1		4.50	4.40	12.85	10.03	8.32	4.02	3.56
B - 2		4.50	4.25	12.85	9.89	7.05	4.02	4.00
B - 3		4.50	3.75	12.85	8.87	7.01	4.02	3.66
C - 1		4.50	4.10	12.85	-	-	4.02	-
C - 2		4.50	3.99	12.85	-	-	4.02	-
C - 3		4.50	3.82	12.85	-	-	4.02	-

(1) 5% of the kernel weight.

(2) See Table XX for identification.

(3) Determinations were carried out in January (time 0), April-May (time 1) and October (time 2).

(4) $\mu\text{eq/g}$ rice, dry basis.

Table XXXIX.- Changes in free amino N content of milled rice during storage.

Storage experiment	Samples (1)	Storage time(2)	Free amino N (3)			
			0	1	2	3
1964-65	II - 1		12.3	11.7	11.7	-
	II - 2		12.3	11.4	10.4	-
	II - 3		12.3	-	10.6	16.0
	II - 4		12.3	10.9	10.3	12.9
	V - 1		6.9	6.6	5.9	-
	V - 2		6.9	6.7	4.4	4.1
	V - 3		6.9	-	5.1	-
	V - 3		6.9	-	5.5	-
	V - 4		6.9	5.6	6.9	5.1
1965-66	II-A-3		10.5	8.9	-	-
	II-B-3		9.5	9.7	9.5	-
	II-C-4		10.9	11.0	-	-
	II-C-1		10.9	10.2	9.6	-
	II-C-2		10.9	11.4	10.6	-
	II-C-3		-	11.6	12.1	-
	IV-A-3		4.1	5.2	3.5	-
	IV-B-2		4.7	-	5.3	-
	IV-B-3		4.7	6.2	-	-
	IV-C-4		3.9	-	4.7	-
	IV-C-1		3.9	-	5.0	-
1966-67	IV-C-2		3.9	4.5	6.3	-
	IV-C-3		3.9	6.5	5.8	-
	A - 1		3.4	-	3.0	-
	A - 2		3.4	-	2.6	-
	A - 3		3.4	-	2.1	-
	B - 1		3.4	-	3.6	-
	B - 2		3.4	-	3.6	-
	B - 3		3.4	-	3.6	-
	C - 1		3.4	-	2.4	-
	C - 2		3.4	-	2.4	-
	C - 3		3.4	-	2.4	-

(1) See Tables XVIII, XIX and XX for identification. (2) Determinations were carried out on December-January (time 0), March (time 1), May-June (time 2) and October (time 3). (3) mg amino N/100 g rice, dry basis.

The magnitude of FAN changes depends on milling degree and storage conditions, as it generally happens with other compositional changes. Low milling degrees, and high moisture contents and temperature favour the changes. At -20°C and $+5^{\circ}\text{C}$ these were non

Table XL.- Effects of storage on free amino N content of milled rice: Comparison of changes in outer layer, nucleus and entire kernel.

Storage experiment	Samples (1)	Storage time(2)	Free amino N content (4)					
			Entire kernel		Outer layer (3)		Nucleus	
			0	1	0	1	0	1
1965-66	II-C-4		10.9	9.8	40.0	46.0	4.5	4.3
	II-C-1		10.9	9.6	40.0	42.1	4.5	4.6
	II-C-2		10.9	10.6	40.0	35.9	4.5	6.5
	II-C-3		10.9	10.9	40.0	23.8	4.5	7.6
	II-C-3		10.9	10.9	40.0	23.8	4.5	7.6
	II-B-3		9.5	9.6	40.0	28.0	4.5	6.9
	II-A-3		10.5	10.1	40.0	36.0	4.5	5.5
	IV-C-4		3.9	4.7	16.1	17.7	3.2	3.2
	IV-C-1		3.9	5.0	16.1	16.9	3.2	3.4
	IV-C-2		3.9	6.3	16.1	13.4	3.2	4.8
	IV-C-3		3.9	5.8	16.1	11.6	3.2	5.3
	IV-C-3		3.9	5.8	16.1	11.6	3.2	5.3
	IV-B-3		4.7	5.7	16.1	12.6	3.2	4.5
	IV-A-3		4.1	4.3	16.1	13.1	3.2	3.1
1966-67	A - 1		3.4	3.0	25.1	24.6	2.5	2.4
	A - 2		3.4	2.6	25.1	24.7	2.5	1.8
	A - 3		3.4	2.1	25.1	21.5	2.5	2.1
	B - 1		3.4	3.6	25.1	23.9	2.5	2.3
	B - 2		3.4	3.6	25.1	23.6	2.5	1.9
	B - 3		3.4	3.6	25.1	15.9	2.5	3.0

(1) See Tables XIX and XX for identification. (2) Determinations were carried out: a) experiment 1965-66: on December-January (time 0), and on May (time 1); b) experiment 1966-67: on February (time 0) and on May-June (time 1). (3) 10% of the kernel weight in 1965-66 experiment and 5% of the kernel weight in 1966-67 experiment. (4) mg amino N/100 g rice, dry basis.

important. At + 25°C FAN decreased in samples with high moisture content. At + 35°C changes were significant in samples with normal and high moisture content.

That the loss of FAN might be due to Maillard-type non enzymatic browning reaction was suggested by the fact that the decrease in FAN appeared to be associated with development of browning (compare Tables XXXIX - XL and XXII - XXIII). It appears that consumption of amino acids is not the only type of changes. As quoted by Zeleny (270),

proteolytic enzymes in grain, and in organisms associated in grain, hydrolyse the proteins into polypeptides and finally into amino acids. These reactions ordinarily proceed very slowly and are not readily measurable until the grain has reached an advanced stage of deterioration(318). It has been reported (320) that prolonged storage of wheat and milled wheat products decreases protein N and increases free amino acid N.

It has been seen above that hermetic storage of milled rice did not bring about significant changes in free amino N in none of the three storage experiments. Perhaps, the state of deterioration was not advanced enough. However, it should be mentioned that rice sample II-5 (7.6% milling degree, 14.6% moisture content, held at + 25°C) stored in an aerated cabinet, deteriorated rapidly and, simultaneously, increased its free amino N from 12 to 39 mg/100 g rice, dry basis.

3. ENZYMES

As shown by data given in Table XLI, storage results in decrease activity of both three enzymes. Similar results have been reported in related papers for amylase (or alpha- and beta-amylase) activity of polished rice (entire kernel) (321)(323), brown rice (92)(273) (224)(225)(323) and rough rice (96)(28). Alpha-amylase levels were very low and changes were appreciated with difficulty. In the entire kernel, only B-3 and C-3 samples (14.3% and 15.7% M.C., at + 35°C) showed a significant change. In the outer layer, with higher alpha-amylase levels, significant changes were detected in all samples examined, even in rice with 13.0% M.C., held at + 5°C. Changes in beta-amylase activity were remarkable and although variations were of greater magnitude in the outer layer than in the nucleus or the entire kernel, changes in the latter were sufficiently large to be detected in almost all of the samples. High temperature and moisture content accelerate the loss of enzymic activities. Changes in proteolytic activity were negligible in the entire kernel but significant in the outer layer as it was the case with alpha-amylase. Losses were not, however, remarkable except in samples stored under the most severe conditions.

4. LIPIDS

4.1. Total lipids.

Results from 1965-66 experiment showed that total lipids content of milled rice stored during ten months under different severe conditions remained unchanged both in outer and inner layers (Table XLII and Fig. 20). Results from the 1966-67 storage experiment confirmed these data (Table XLIII).

4.2. Free fatty acids (FFA), neutral fats (NF) and phospholipids (P). (Tables XLII and XLIII and Fig. 20).

4.2.a. Changes in the entire kernel. FFA increased and NF and P decreased with storage. Similar results were reported by other workers (11) for polished rices stored in non hermetic conditions.

Table XLI. - Effects of storage of milled rice on alpha-amylase, beta-amylase and proteolytic activities of entire kernel, outer layer and nucleus (1966-67 experiment).

Samples ⁽¹⁾	Alpha-amylase activity ⁽²⁾			Beta-amylase activity ⁽³⁾			Proteolytic activity ⁽⁴⁾	
	Entire kernel	Outer layer ⁽⁵⁾	Nucleus	Entire kernel	Outer layer ⁽⁵⁾	Nucleus	Entire kernel	Outer layer ⁽⁵⁾ Nucleus
A	0.11	1.03	0.07	-	-	-	0.97	6.03 0.63
A - 1	0.11	0.65	0.07	44.89	223.81	31.26	1.12	5.77 0.61
A - 2	0.13	0.47	0.08	42.17	216.00	32.13	1.08	5.36 0.57
A - 3	0.11	0.86	0.06	27.11	148.06	19.43	0.91	4.22 0.53
B - 1	-	-	-	-	-	-	-	- -
B - 2	0.14	0.62	0.09	25.16	192.22	11.83	1.12	4.23 0.61
B - 3	0.06	0.38	0.03	20.34	88.20	15.88	0.77	3.89 0.44
C - 1	-	-	-	36.87	-	-	-	- -
C - 2	0.13	-	-	30.34	-	-	0.76	- -
C - 3	0.06	-	-	18.63	-	-	-	- -

(1) See Table XX for identification. Sample A is A-1 analyzed in January. Determinations in other samples were carried out in May-June. (2) S. K. B. units/g. rice, d.b. (3) mg. maltose/g. rice, d.b. (4) Hemoglobin units/g. rice, d.b. (5) 5% of the kernel weight.

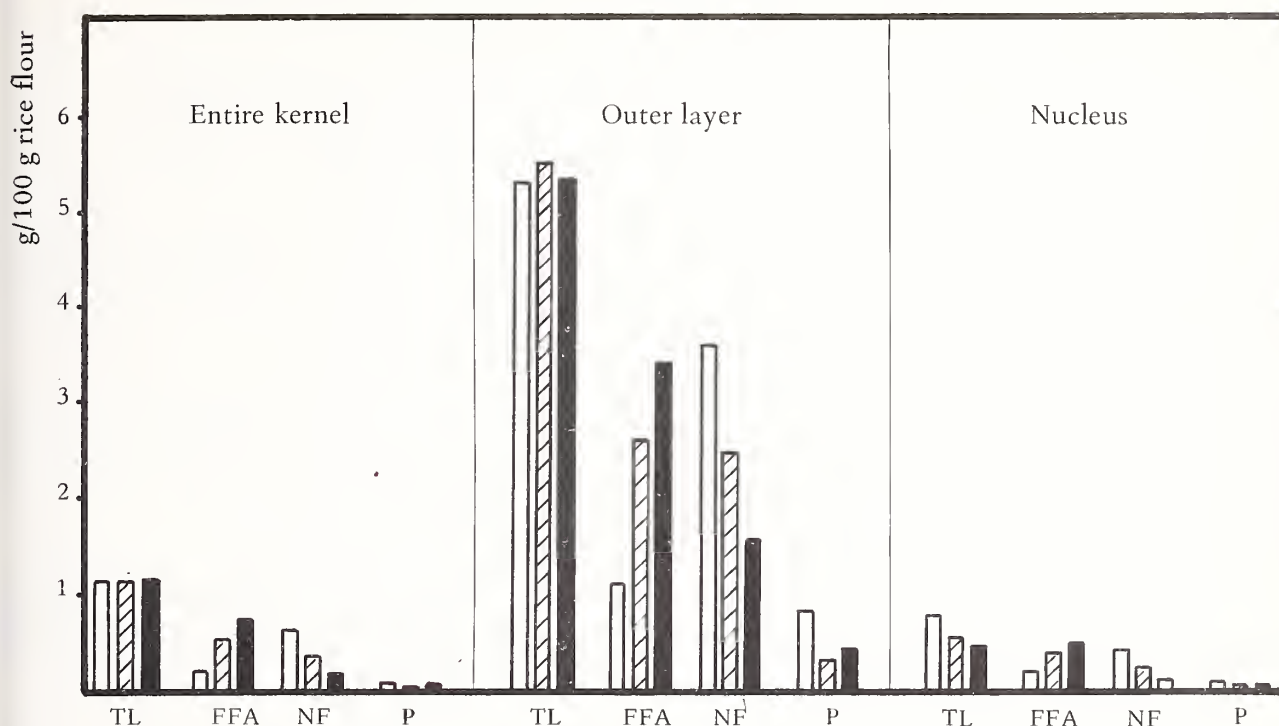


Fig. 20.- Changes in lipids of milled rice during air-tight storage: comparison of changes in outer layer, nucleus and entire kernel.

— Before storage
 — After three months
 — After five months

TL : Total lipids
 FFA: Free fatty acids
 NF : Neutral fats
 P : Phospholipids

— Milling degree: 7.7 %
 — Moisture content: 13.7 %
 — Storage temperature: 35°C
 — Outer layer: 10 % of kernel weight

FFA and NF underwent significant changes. Phospholipids, although showed a definite tendency to decrease, underwent small variation. Before storage, NF fraction was the component predominant in amount of total lipids of rice. In rices of 1965-66 experiment (Table XLII) NF represented about the 60%; FFA accounted for the 25% and P for the remaining 15%. Storage resulted in significant changes. After three months FFA amounted to 55% of TL, and NF and P decreased to 40% and 5% respectively. After then months, FFA increased up to 70%, and NF decreased proportionally whereas P did not show a significant change.

Both under- and well milled rices showed the same trend of changes. When considering results on a rice basis -i.e., g lipid fraction/100 g rice- changes were dependent upon milling degree, moisture content and temperature, and their rate increased first and decreased with prolonged storage time. On a lipid basis, changes in under- and well milled rices were of some magnitude: from each gram of fat approximately the same quantity of FFA was released.

Table XLII.- Changes in lipid and lipid fractions content of milled rice during storage: Comparison of changes in outer layer, nucleus and entire kernel (1965-66 experiment).

Storage time(1)	Total lipids (2)(3)								
	Entire kernel			Outer layer (4)			Nucleus (5)		
	0	1	2	0	1	2	0	1	2
Undermilled rice (6)	1.18	1.17	1.19	5.28	5.40	5.30	0.71	0.66	0.61
Well milled rice (7)	0.70	0.69	0.69	2.24	2.23	2.37	0.52	0.49	0.47
	Free fatty acids (2)								
	0	1	2	0	1	2	0	1	2
Undermilled rice (6)	0.29	0.64	0.84	1.05	2.64	3.36	0.20	0.40	0.46
Well milled rice (7)	0.15	0.38	0.48	0.44	1.17	1.47	0.12	0.28	0.29
	Neutral fats (2)								
	0	1	2	0	1	2	0	1	2
Undermilled rice (6)	0.73	0.46	0.28	3.52	2.44	1.51	0.42	0.23	0.12
Well milled rice (7)	0.43	0.26	0.15	1.54	0.96	0.63	0.31	0.17	0.11
	Phospholipids (2)								
	0	1	2	0	1	2	0	1	2
Undermilled rice (6)	0.16	0.06	0.08	0.72	0.31	0.43	0.09	0.03	0.03
Well milled rice (7)	0.10	0.05	0.06	0.25	0.10	0.26	0.09	0.04	0.07

(1) Determinations were carried out on December (time 0), March (time 1) and October (time 2).
 (2) g/100 g rice flour, d.b. (3) 2:1 chloroform:methanol extractable lipids. (4) 10% of the kernel weight. (5) By calculation. (6) 7.7% of milling, 13.7% M.C., +35°C. (7) 12.0% of milling, 14.2% M.C., +35°C.

4.2.b. Changes in outer layer compared to those in nucleus and entire kernel. In outer layer FFA increased and NF and P decreased, as it happened in the entire kernel. The nucleus showed the same trend of changes. On a lipid basis, results showed that changes in FFA, NF and P proportions do not depend on location of the fat material within the kernel. Same amount of FFA was formed per gram of lipid in any part of the rice kernel. However, when considering results on a rice basis the extent of changes was greatly influenced by the deepness of the layer. As it can be seen in Fig. 20, changes were much greater in amount in the outer than in the inner region of the kernel. During the first three months, FFA content of outer layer (undermilled rice) increased 1.59 g/100 g flour whereas that of the nucleus only did 0.20 g. According to these data, the 50% of total changes in the kernel took place in the outer layer of only about 0.1 mm thick.

From these results it follows that average composition data of the entire kernel do not reveal the actual condition of stored rice lipids. The large variations occurring in outer layer are diluted by the rather unchanged nucleus. Lipid changes, as measured in the entire kernel, are slow and small, being difficult to be detected in the early stages of deterioration. Separate consideration of changes in outer layer affords a more real information. On the other

Table XLIII.- Changes in lipid and lipid fractions content of milled rice during storage: Comparison of changes in outer layer, nucleus and entire kernel (1966-67 experiment).

Samples (1)	Storage time(6)	Total lipids (2)(3)					
		Entire kernel		Outer layer (4)		Nucleus (5)	
		0	1	0	1	0	1
A - 1		0.66	0.67	4.44	4.43	0.45	0.47
A - 2		0.66	0.67	4.44	4.47	0.45	0.48
A - 3		0.66	0.66	4.44	4.41	0.45	0.46
B - 2		0.66	0.61	4.44	4.41	0.45	0.41
C - 2(7)		0.66	0.64	-	-	-	-
Free fatty acids (2)							
A - 1		0.21	0.20	1.34	1.32	0.15	0.16
A - 2		0.21	0.29	1.34	1.61	0.15	0.22
A - 3		0.21	0.38	1.34	2.14	0.15	0.28
B - 2		0.21	0.36	1.34	2.30	0.15	0.25
C - 2(7)		0.21	0.45	-	-	-	-
Neutral fats (2)							
A - 1		0.38	0.39	2.53	2.57	0.26	0.27
A - 2		0.38	0.32	2.53	2.36	0.26	0.22
A - 3		0.38	0.21	2.53	1.62	0.26	0.13
B - 2		0.38	0.20	2.53	1.74	0.26	0.12
C - 2(7)		0.38	0.14	-	-	-	-
Phospholipids (2)							
A - 1		0.07	0.08	0.57	0.54	0.04	0.04
A - 2		0.07	0.06	0.57	0.49	0.04	0.04
A - 3		0.07	0.08	0.57	0.64	0.09	0.05
B - 2		0.07	0.05	0.57	0.37	0.04	0.04
C - 2(7)		0.07	0.05	-	-	-	-

(1) See Table XX for identification.

(2) 2:1 chloroform: methanol extractable lipids.

(3) g lipids/100 g rice flour, d.b.

(4) 5% of the kernel weight.

(5) By calculation.

(6) Determinations were carried out on Dec-Jan. (time 0) and May-June (time 1).

(7) Outer layer could not be removed because of breakage of kernels.

Table XLIV.- Changes in lipid composition of milled rice during storage: Comparison of changes in outer layer, nucleus and entire kernel (1965-66 experiment).

Storage time(1)	Free fatty acids (2)								
	Entire kernel			Outer layer (3)			Nucleus (4)		
	0	1	2	0	1	2	0	1	2
Undermilled rice (5)	24.76	54.70	69.74	17.50	48.98	63.34	28.17	60.61	74.41
Well milled rice (6)	22.32	55.33	70.34	19.96	52.35	65.39	23.08	57.14	64.33
	Neutral fats (2)								
	0	1	2	0	1	2	0	1	2
Undermilled rice (5)	61.87	39.75	23.31	68.81	45.19	28.52	59.15	34.85	19.87
Well milled rice (6)	62.02	37.58	20.72	68.76	42.95	30.13	59.61	34.69	25.62
	Phospholipids (2)								
	0	1	2	0	1	2	0	1	2
Undermilled rice (5)	13.37	5.55	6.95	13.69	5.83	8.14	12.68	4.54	5.72
Well milled rice (6)	15.66	7.09	8.94	11.28	4.70	4.48	17.31	8.17	10.05

(1) Determinations were carried out on December (time 0), March (time 1) and October (time 2).
 (2) g/100 g lipids. (3) 10% of the kernel weight. (4) By calculation. (5) 7.7% of milling, 13.7% M.C., +35°C. (6) 12.0% of milling, 14.2% M.C., +35°C.

hand, this is of major interest if it is borne in mind the predominant influence of the outer layer in rice behavior during cooking and processing.

4.3. Fatty acid composition of lipid fractions

4.3.1. Free fatty acids (FFA). Fatty acid composition of FFA fraction changed during storage (Table XLVI). The proportion of palmitic acid and that of linoleic acid decreased whereas that of oleic acid increased. These results were in agreement with those reported by other workers (11)(184) for Japanese and American rice varieties stored under conditions different to those studied here. The trends of fatty acid composition changes were similar in undermilled and in well milled rices.

The fatty acid composition of the FFA fraction changed both in outer layer and in nucleus. In the former, the proportion of palmitic acid decreased and those of oleic and linoleic acids increased; the pattern of changes was therefore different from that found in the entire kernel. On the contrary, changes in the nucleus were as those in the entire kernel.

Data on the influence of temperature and of moisture content on the fatty acid composition changes during storage of milled are given in Table XLVII. It can be seen that the higher the level of both parameters, the more rapid the changes were.

Table XLV.- Changes in lipid composition of milled rice during storage: Comparison of changes in outer layer, nucleus and entire kernel (1966-67 experiment).

Samples (1)	Storage time(5)	Free fatty acids (2)					
		Entire kernel		Outer layer (3)		Nucleus (4)	
		0	1	0	1	0	1
A - 1		31.31	29.85	30.03	29.79	33.33	34.04
A - 2		31.31	43.29	30.03	36.17	33.33	45.83
A - 3		31.31	56.93	30.03	48.55	33.33	60.87
B - 2		31.31	58.00	30.03	52.15	33.33	60.97
C - 2 (6)		31.31	70.31	-	-	-	-
		Neutral fats (2)					
		Entire kernel		Outer layer (3)		Nucleus (4)	
		0	1	0	1	0	1
A - 1		58.63	58.20	57.16	58.01	57.78	57.44
A - 2		58.63	47.92	57.16	52.71	57.78	45.83
A - 3		58.63	31.35	57.16	37.05	57.78	28.26
B - 2		58.63	32.78	57.16	39.45	57.78	29.26
C - 2 (6)		58.63	21.87	-	-	-	-
		Phospholipids (2)					
		Entire kernel		Outer layer (3)		Nucleus (4)	
		0	1	0	1	0	1
A - 1		10.06	11.95	12.81	12.20	8.89	8.44
A - 2		10.06	8.79	12.81	11.12	8.89	8.34
A - 3		10.06	11.71	12.81	14.39	8.89	10.87
B - 2		10.06	8.22	12.81	8.40	8.89	9.77
C - 2 (6)		10.06	7.82	-	-	-	-

(1) See Table XX for identification.

(2) g/100 g lipids.

(3) 5% of the kernel weight.

(4) By calculation.

(5) Determinations were carried out on Dec.-Jan. (time 0) and May-June (time 1).

(6) Outer layer could not be removed because of breakage of kernels.

Table XLVI.- Changes in the fatty acid composition of free fatty acid fraction of milled rice during storage: Comparison of changes in outer layer, nucleus and entire kernel (1965-66 experiment).

Fatty Acid(3)	Storage time(4)	Undermilled rice (1)								
		Entire kernel			Outer layer (5)			Nucleus (6)		
		0	1	2	0	1	2	0	1	2
Lauric		Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr
Myristic		1.3	0.3	0.4	0.8	0.2	0.3	1.5	0.3	0.5
Palmitic		23.3	17.7	18.3	22.9	17.1	17.0	23.5	18.1	20.8
Palmitoleic		Tr	Tr	Tr	0.3	Tr	Tr	Tr	Tr	Tr
Stearic		1.4	1.0	0.7	1.6	1.0	0.4	1.0	0.9	1.1
Oleic		27.6	36.5	37.2	35.6	39.1	40.5	23.0	34.3	37.4
Linoleic		44.0	43.8	42.5	37.0	41.5	41.3	48.5	45.7	39.4
Linolenic		2.4	0.8	0.8	1.8	1.1	0.6	2.5	0.6	0.9
Arachidic		-	Tr	Tr	Tr	Tr	Tr	-	Tr	Tr

		Well milled rice (2)								
		Entire kernel			Outer layer (5)			Nucleus (6)		
		0	1	2	0	1	2	0	1	2
Lauric		Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	-
Myristic		1.0	0.3	0.5	0.9	0.2	0.2	0.8	0.4	-
Palmitic		20.8	17.9	18.1	23.0	17.4	16.8	18.3	18.0	-
Palmitoleic		Tr	Tr	0.6	0.3	Tr	Tr	Tr	Tr	-
Stearic		1.5	1.0	0.6	1.7	0.8	0.8	0.8	1.1	-
Oleic		23.6	35.8	32.0	31.4	37.8	39.5	21.7	35.3	-
Linoleic		51.3	43.8	47.8	40.8	42.9	41.8	56.7	43.8	-
Linolenic		1.8	1.2	1.0	2.0	0.8	0.8	1.7	1.4	-
Arachidic		-	Tr	Tr	Tr	Tr	Tr	-	Tr	-

(1) 7.7% of milling; 13.7% M.C.; +35°C

(2) 12.0% of milling; 14.2% M.C.; +35°C

(3) g fatty acid/100 g free fatty acids fraction.

(4) Determinations were carried out at the beginning of the storage (time 0) and after (time 1) and ten months (time 2).

(5) 10% of the kernel weight.

(6) By calculation.

Table XLVII.- Changes in the fatty acid composition of lipid fractions (1966-67 experiment).

Sample Fatty (1) acid(2)(3)		FREE FATTY ACID FRACTION								
		a) Influence of temperature								
		12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0
Entire kernel	A-1	Tr	0.42	18.72	Tr	1.11	25.96	51.72	2.07	Tr
	A-2	Tr	0.61	20.45	Tr	0.88	29.86	47.11	1.09	Tr
	A-3	Tr	0.26	20.24	Tr	0.65	35.70	42.78	0.39	Tr
Outer layer(4)	A-1	Tr	0.21	18.32	Tr	0.90	34.74	41.06	2.07	Tr
	A-2	Tr	0.25	21.33	Tr	0.62	38.53	38.22	1.05	Tr
	A-3	Tr	0.19	19.38	Tr	0.66	40.53	39.26	0.36	Tr
		b) Influence of moisture content								
		12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0
Entire kernel	A-2	Tr	0.61	20.45	Tr	0.88	29.86	47.11	1.09	Tr
	B-2	Tr	0.20	17.28	Tr	1.09	34.51	45.88	1.04	Tr
	C-2	Tr	0.50	16.92	Tr	1.50	39.78	41.22	0.08	Tr
Outer layer(4)	A-2	Tr	0.25	21.33	Tr	0.62	38.53	38.22	1.05	Tr
	B-2	Tr	0.10	17.87	Tr	0.72	40.22	40.23	0.86	Tr
		NEUTRAL FAT								
Sample Fatty (1) acid(2)(3)		a) Influence of temperature								
		12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0
Entire kernel	A-1	Tr	0.53	18.17	Tr	1.28	33.47	45.10	1.45	Tr
	A-2	Tr	0.40	19.10	Tr	0.74	34.72	44.56	0.48	Tr
	A-3	Tr	0.35	20.52	Tr	1.22	33.15	43.86	0.90	Tr
Outer layer(4)	A-1	Tr	0.53	16.56	Tr	1.79	39.76	40.09	1.27	Tr
	A-2	Tr	0.14	18.13	Tr	0.73	40.54	39.95	0.51	Tr
	A-3	Tr	0.09	17.82	Tr	0.87	39.40	41.42	0.40	Tr
		b) Influence of moisture content								
		12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0
Entire kernel	A-2	Tr	0.40	17.10	Tr	0.74	37.72	48.56	0.48	Tr
	B-2	Tr	0.52	18.06	Tr	1.15	33.29	45.99	0.99	Tr
	C-2	Tr	0.70	17.50	Tr	1.35	33.90	46.55	Tr	Tr
Outer layer(4)	A-2	Tr	0.14	18.13	Tr	0.73	40.54	39.95	0.51	Tr
	B-2	Tr	0.11	16.85	Tr	1.65	38.53	41.86	1.00	Tr

(1) See Table XX for identification.

(2) g fatty acid/100 g free fatty acids fraction.

(3) The number to the left of the colon denotes the length of the carbon chain, and the number to the right of the colon represents the number of double bonds in the fatty acids.

(4) 5% of the kernel weight.

4.3.2. Neutral fats (NF). (Table XLVII). Storage of rice samples under quite different conditions did not result in significant changes of the fatty acid composition of the NF fraction. Similar results have been reported by Yasumatsu and Moritaka (11).

4.3.3. Phospholipids. Results obtained were not satisfactory. Reproducibility was not good. It seems that the small amount of phospholipids in rice and the error of the procedure (mainly due to the low specificity of the fractionation step which is based on the insolubility of phospholipids in acetone) are the main causes.

4.4. Chemical characteristics of lipids

4.4.1. Fat acidity. Results are presented discussing the influence of a) milling degree, b) moisture content and c) temperature of storage.

a) Influence of milling degree. (Table XLVIII). Fat acidity increased with storage time. When considering results on a rice basis the following facts are observed: 1) Acidity increases faster in the undermilled than in the well milled rices and 2) acidity changes take place in the outer layer at a faster rate than in the nucleus. The trends of changes are, therefore, similar to those found in the free fatty acids content, commented previously.

Results given on a fat basis indicate that unlike acidity in rice, fat acidity undergoes changes at the same rate practically in undermilled and in well milled rices. This is in agreement with the changes found in fat composition. However, unlike the changes in FFA content of lipids, the rate of changes in fat acidity appears to be faster in the outer layer than in the nucleus. Nevertheless, data from a latter experiment did not confirm this difference. This apparent divergency is not well understood. It seems to indicate that factors associated with the "surface" of the kernel (like microflora) may influence deterioration rate. This would explain that the rate of changes in acidity (mg KOH/g fat) is the same in outer layer of under- and well milled rices, despite the difference in deepness which, in turns, means difference in chemical composition.

b) Influence of moisture content. (Table XLIX). In the entire kernel, acidity increased with moisture content; in advanced state of rice deterioration, acidity reached a maximum. Acidity changes in the outer layer were of interest. After six months of storage, as well as after ten months, the samples with lower moisture content showed the highest acidity values. The lack of data for shorter storage periods did not allow us to plot the continued curve of variation. However, it seems clear that acidity, after reaching a maximum value, decreased. Similar changes were observed with other samples (see below).

Changes in the nucleus were similar to those in the outer layer but much more slow. As a result, when acidity in the outer layer reaches the maximum and begins to decrease, acidity in the nucleus may still be increasing.

Our results were in agreement with those obtained by Hunter et al., (284) when studying the development of free fatty acids in laboratory hulled brown rice of different moisture contents stored at $\pm 25^{\circ}\text{C}$. After the first 150 days of storage, the free fatty acids in oil (as measured by acid titration) did not show any further increase; in fact, slightly

Table XLVIII.- Changes in fat acidity during storage of undermilled and well milled rices (1965-66 experiment).

Samples		mg KOH/100 g rice			mg KOH/g lipids		
		December	March	October	December	March	October
II-B-3 ⁽¹⁾	Entire kernel	47.6	91.1	124.8	40.3	78.5	104.8
	Outer layer(3)	247.3	-	756.5	46.8	-	142.7
	Nucleus	26.9	-	50.3	37.8	-	82.4
IV-B-3 ⁽²⁾	Entire kernel	17.9	45.5	60.9	25.5	55.9	88.2
	Outer layer(3)	108.4	-	347.1	48.3	-	146.4
	Nucleus	6.8	-	26.0	13.0	-	55.3

(1) Undermilled rice, with 13.7% M.C., stored at +35°C.

(2) Well milled rice, with 14.2% M.C., stored at +35°C.

(3) 10% of the kernel weight.

Table XLIX.- Changes in fat acidity during storage of milled rice (1965-66 experiment).

Samples (1)	Storage time(3)	Influence of moisture content (1)									
		Entire kernel				Outer layer (4)			Nucleus		
		0	1	2	3	0	2	3	0	2	3
✓											
II-A-3		46.8	66.1	89.6	97.9	57.5	160.7	157.0	38.3	42.1	64.2
II-B-3		40.3	77.2	96.3	105.8	46.8	145.8	143.3	37.9	63.1	70.8
II-C-3		57.8	101.9	100.4	101.1	62.8	118.7	108.9	31.4	72.8	60.8
Influence of temperature (1)											
II-C-4		57.8	58.4	56.6	55.8	62.8	90.2	91.4	31.4	30.3	30.1
II-C-1		57.8	59.3	59.7	83.3	62.8	97.1	90.8	31.4	30.3	62.5
II-C-2		57.8	86.2	95.9	102.7	62.8	181.1	122.0	31.4	54.1	-
II-C-3		57.8	101.9	100.4	101.1	62.8	118.7	108.9	31.4	72.8	60.8
IV-C-4		39.5	37.2	38.9	39.4	70.1	76.9	75.5	19.2	20.2	21.7
IV-C-1		39.5	39.5	39.3	47.1	70.1	80.7	51.1	19.2	19.2	34.8
IV-C-2		39.5	60.3	75.1	78.3	70.1	174.5	140.4	19.2	27.9	44.8
IV-C-3		39.5	65.0	87.7	85.3	70.1	183.2	108.0	19.2	42.9	50.2

(1) Results are given as mg KOH/g lipids.

(2) See Table XIX for identification.

(3) Determinations were carried out on December (0), March (1), June (2) and October (3).

(4) 10% of the kernel weight.

lower values were found between the 150 and 300 days. Similar results have also been reported for parboiled rice (286). It has also been reported (209) that during storage of rice polishings for 17 months the acid number of oil increased to a maximum and then showed a gradual decrease till the end. Similar trends have been found in acidity during storage of rough rice (288).

Iwasaki and Tani (273) have recently reported that one year storage of brown rice in air results in lower fat acidity than in N_2 atmosphere. It seems probable that fat acidity in the former reached a maximum level and decreased afterwards at the time determination.

The information obtained may be of interest in connection with the use of acidity as an index of deterioration in rice (and, likewise, in other cereal grains). Fat acidity has received much attention since high correlation coefficients have been found between it and the percentage of damaged kernels - loss of viability. However, despite high correlations have been found, the scatter of the experimental points about the regression line has been frequently quite appreciable. It might be a point to study whether or not the different changes of acidity in the outer layer and in the nucleus could be responsible for it. The outer layer or the germ itself may undergo changes quite different from those measured in the entire kernel.

c) Influence of storage temperature. Table XLIX reports data of fat acidity of under- and well milled rices with 15.6/15.5% moisture content, stored during ten months at different temperatures. Changes were proportional to temperature. The induction period was longer when storage temperatures were lower. At $-20^{\circ}C$ changes did not appear; at $+5^{\circ}C$ changes appeared after six months. Rices held at $+25^{\circ}C$ and $+35^{\circ}C$ showed an increase in fat acidity before the third month. Similar effects of temperature on acidity have been reported for brown rice (224) (225) (272) and rough rice (272).

Acidity showed similar trends of changes in the nucleus and in the outer layer; changes in the latter were faster, induction periods appeared to be shorter. Slight but significant increases took place in the outer layer even in samples stored at low temperatures. Results given in Table XLIX also show that fat acidity increased until reaching a maximum and decreased afterwards.

4.4.2. Iodine value. Table L reports data obtained at the beginning and after three months of storage (1966-67 experiment). Storage resulted in significant decreases in iodine value, these being influenced by holding conditions. Changes in lipids of the entire kernel were negligible in samples with 13.0% and 14.3% M.C., stored at $+5^{\circ}$ and $+25^{\circ}C$. At $+35^{\circ}C$ changes were remarkable.

Changes in the outer layer were greater than in the entire kernel. They also were measurable earlier. With the only exception of rice with 13.0% M.C., held at $+5^{\circ}C$, changes were detected in all samples.

4.4.3. Peroxide value. Peroxide values (Table L) remained extremely low in all the samples examined. Even though values for lipids of the outer layer increased somewhat, final levels also were low. Small increases have also been reported previously for brown rice (214) (92) (273), milled rice (92) and parboiled rice (92) (286) (137).

Table L.- Changes in iodine value and peroxide value of lipids of milled rice during storage:
Comparison of changes in outer layer, nucleus and entire kernel (1966-67 experiment).

Samples (1)	Storage time(4)	Iodine value (2)					
		Entire kernel		Outer layer (3)		Nucleus	
		0	1	0	1	0	1
A - 1		102.1	101.4	120.9	120.0	96.2	95.6
A - 2		102.1	99.4	120.9	107.8	96.2	95.3
A - 3		102.1	89.0	120.9	95.6	96.2	80.6
B - 1		102.1	98.0	120.9	110.9	96.2	96.0
B - 2		102.1	100.9	120.9	100.7	96.2	97.7
B - 3		102.1	91.9	120.9	94.6	96.2	77.9
C - 1		-	95.3	120.9	-	-	-
C - 2		-	83.0	120.9	-	-	-
C - 3		-	76.7	120.9	-	-	-
Peroxide value (5)							
A - 1		0.52	0.49	0.70	0.64	0.31	0.28
A - 2		0.52	0.51	0.70	0.89	0.31	0.30
A - 3		0.52	0.65	0.70	2.86	0.31	0.35
B - 1		0.52	0.70	0.70	0.60	0.31	0.41
B - 2		0.52	0.79	0.70	1.05	0.31	0.69
B - 3		0.52	0.70	0.70	0.65	0.31	0.42
C - 1		0.52	0.81	-	-	-	-
C - 2		0.52	1.04	-	-	-	-
C - 3		0.52	0.98	-	-	-	-

(1) See Table XX for identification. (2) g I₂/100 g lipids. (3) 5% of the kernel weight. (4) Determinations were carried out on February (time 0) and May-June (time 1). (5) meq. peroxide/kg lipids.

The low levels found, and the fact that values in samples with 14.3% and 15.7% M. C., held at +35°C were lower than those for corresponding samples held at +25°C, suggest that accelerated decomposition takes place soon after peroxide formation. This has been reported to occur in parboiled rice.

4.4.4. TBA test. Presence of malonaldehyde was not detected in any sample after three months of storage. Additional tests carried out with more deteriorated samples were negative.

IV. EFFECTS OF STORAGE ON MICROFLORA OF MILLED RICE

1. Bacteria and mold-and yeast counts.

Storage decreased the number of microorganisms in all samples (Table LI). Bacteria and mold-yeasts counts showed parallel trends and similar percentage losses. Differences in moisture content (samples ranged from 13.0% to 15.7% M.C.) did not result in significant differences in the rate of changes. However, storage temperature had a definite influence: both bacteria and mold-yeasts counts were much lower in samples held at high than at low temperatures.

These results compare fairly well with related data reported by various authors on the effects of storing rough rice (138)(237)(135).

Parallel to the gradual decrease in the total number of microorganisms, an increase of CO₂ in intergranular atmosphere was registered (see Table XXIX). CO₂ concentration reached high levels very soon in samples stored under severe conditions; the sharp bacteria and molds counts decreases are largely, if not mainly, due to it. Correlation between microbial and CO₂ changes does not hold however in all cases. In samples held at +5°C, CO₂ concentration remained practically constant throughout the experiment (particularly in samples with 13.0% and 14.3% moisture content) whereas the number of microorganisms decreased significantly.

2. Mold-infested kernels.

Storage changes in % kernels from which mold colonies can be obtained were followed in the samples of 1966-67 experiment (Table LI), using the procedure described by Schroeder (322). Results did not show a trend of changes common to all rices although a tendency to increase was observed in most samples.

Whereas the total counts, with decreasing values, do not look to supply a realistic idea of the condition of samples along storage, the percentage of mold-infested kernels, whether confirmed to increase with storage time, might afford a more actual evaluation. Therefore, a simple storage test was undertaken. Recently milled rice (7.2% milling degree), with 14.1% moisture content, was packed in sterilized glass bottles, hermetically sealed, and stored in the dark at +25°C. Samples were analysed at periodical intervals. The results, illustrated in Fig. 21, showed that percent mold-infested kernels increased somewhat at the beginning of storage but after 45 days decreased with storage time. Bacteria and molds counts decreased as time of storage increased, confirming previous results (Table L).

3. Types and proportion of bacteria and molds.

Data given in Table LII show variation in types and proportion of bacteria and molds of rice from January to June-July. Bacteria: Predominant in amount at the beginning of storage were "Xantomonas" (35%) and "Sarcina" (45%); "Serratia" and different bacilli were also isolated. Storage at +5°C resulted in increased proportion of "Xantomonas" at the three moisture levels investigated, with corresponding decreases in "Sarcina" and bacilli. At higher

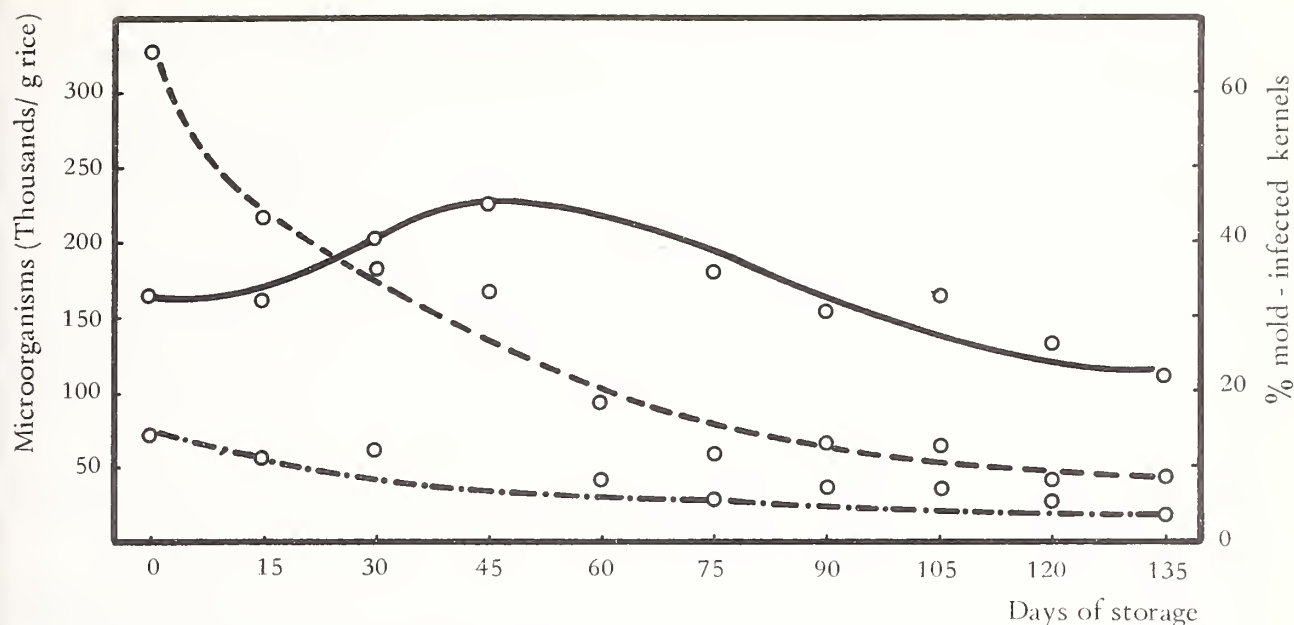


Fig. 21.- Changes in bacteria and mold-yeasts counts and number of mold-infested kernels during air-tight storage of milled rice (14.5 % M.C., + 25°C).

————— Infected kernels
 - - - - - Bacteria
 - . - . - Molds

Table LI.- Changes in bacteria and mold-yeasts counts and number of mold-infested kernels during storage of milled rice (1966-67 experiment).

Samples (1)	Storage time(2)	Bacteria (3)			Molds and yeasts(4)			% infected kernels	
		0	1	2	0	1	2	1	2
A - 1		61	41	5	52	34	14	14	38
A - 2		-	20	6	-	-	1	34	14
A - 3		-	9	0.5	-	9	0.5	22	56
B - 1		-	34	3	-	20	14	12	16
B - 2		-	21	1	-	23	0.5	6	44
B - 3		-	9	0.5	-	8	0.5	10	56
C - 1		-	39	12	-	30	3	38	20
C - 2		-	18	0.5	-	16	0.5	34	70
C - 3		-	14	0.5	-	10	0.5	16	-

(1) See Table XX for identification. (2) Analysis were carried out as follows: time 0, in January; time 1 in March-April; time 2 in June-July. (3) Thousand per gram.

temperatures, the trends were not clear, but it appears that the more severe the conditions were, the bacilli were more abundant in early stages of storage but decreased afterward.

Molds: *Aspergillii* (70%) and *penicillia* (30%) were practically all molds found at the beginning of the experiment. After storage, decreases in *penicillia* and increases in *aspergilli* were noted in most of samples.

In a previous storage experiment (1964-65) the following molds were identified: "*Aspergillus niger*" and "*A. flavus-oryzae*" -predominant in amount-, "*A. ochraceus*", "*A. Terreus*", "*A. wentii*", "*A. glaucus*", "*Penicillium expansum*", "*P. digitatum*", "*P. italicum*" and the genera *Fusarium*, *Alternaria*, *Rhizopus*, *Mucor*, *Absidia* and *Piricularia*.

4. Microflora of commercially milled rice and its variation with date of milling.

Cleaning, milling and conditioning of rice samples used in storage experiments reported above was carried out at the laboratory in order to prepare samples with different milling degree and moisture levels. Interest arose in knowing if the laboratory manipulation of samples could result in a microflora different from that typical in milled rice, limiting therefore the value of the knowledge obtained to the particular conditions of the samples investigated. A supplementary study was planned to determine the typical microflora of milled rice with samples of recently milled rice collected in various local mills. Bacteria and molds and yeasts counts as well as type and proportion of microorganisms were studied.

As microflora of recently milled rice could be dependent on storage of the rough rice previous to milling, samples were collected at different dates during the 1966-67 season. In addition to the knowledge of typical microflora, a general idea on the effects of storage of rough rice on the microflora of freshly milled rice could thus been obtained.

Table LIII reports the results found. Total counts differed significantly from mill to mill and from date to date. Initial values ranged from 90 to 416 thousands bacteria/g rice and 413 to 34 thousands molds/g. Data obtained in previous studies for rice samples prepared at the laboratory were comprised within these ranges or near the lower limits. Lower bacteria and mold counts were obtained some months after harvest than at the beginning of the season. The same trend has been noted in previous studies with air-tight stored milled rices.

As it could be expected, there were significant differences among mills in the total counts of freshly harvested rice. Nevertheless, the tendency to decrease with storage time was general.

Regarding type and proportion of microorganisms present, the results showed the following: a) Bacteria. In general "*Xantomonas oryzae*" was the bacteria predominant; "*Micrococcus albus*" appeared in higher proportion in few samples. Other microorganisms found were "*Serratia marcencens*" and some Bacilli. In samples milled six-nine months after harvest, an increased proportion of *Xantomonas* was found, it accounting in some samples for practically all bacteria present. b) Molds. Most of the molds found were of the *Penicillii* and *Aspergillus* genera. "*A. flavus oryzae*" and "*A. niger*" were the species predominant in

Table LII. - Effects of storage on kinds of microorganisms in milled rice.

Samples (1)	Storage time(2)	Bacteria						Molds																	
		Xantomonas		Sarcina		Serratia		Bacilli		Penicillium		Aspergillus		Alternaria		Rhizopus									
		(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)								
		0	1	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2			
A - 1		35	80	50	45	20	30	1	0	0	15	0	20	30	15	10	70	85	90	0	0	0	0		
A - 2		-	35	68	-	45	20	-	1	12	-	15	0	-	30	40	-	70	60	-	0	0	0		
A - 3		-	40	100	-	30	0	-	0	0	-	30	0	-	15	0	-	85	100	-	0	0	0		
B - 1		-	85	70	-	7	10	-	3	0	-	5	20	-	0	0	-	85	80	-	15	20	-	0	0
B - 2		-	60	90	-	5	10	-	5	0	-	30	0	-	15	20	-	85	80	-	0	0	-	0	0
B - 3		-	35	100	-	25	0	-	0	0	-	45	0	-	15	0	-	70	100	-	0	0	-	15	0
C - 1		-	83	80	-	15	20	-	2	0	-	0	0	-	0	30	-	85	70	-	0	0	-	15	0
C - 2		-	87	40	-	10	60	-	0	0	-	3	0	-	25	25	-	75	75	-	0	0	-	0	0
C - 3		-	10	100	-	30	0	-	0	0	-	60	0	-	15	0	-	85	100	-	0	0	-	0	0

(1) See Table XX for identification. (2) Analysis were carried out on January (time 0), March-April (time 1) and June-July(time 2)

Table LIII. - Microflora of commercially milled rice and its variation with date of milling.

Mill	Date of milling	Bacteria(1)	Molds	Bacteria					Molds			
				Xanthomonas (%)	Micrococcus (%)	Serratia (%)	Bacilli (%)	Aspergillus (%)	Penicillium (%)	Alternaria (%)	Rhizopus (%)	
I	25. XI. 66	415.0	413.0	20	39	41	0	20	79	0	0	
	10. VI. 67	22.0	2.6	60	40	0	0	80	20	0	0	
III	18. I. 67	75.0	12.6	67	37	0	0	-	-	-	-	
	19. VII. 67	5.7	3.6	100	0	0	0	90	10	0	0	
V	1. II. 67	140.0	75.6	39	61	0	0	100	0	0	0	
	20. VII. 67	4.7	2.2	100	0	0	0	100	0	0	0	
VII	17. II. 67	31.1	17.6	40	60	0	0	80	20	0	0	
	28. VI. 67	5.1	3.2	100	0	0	0	100	0	0	0	
VIII	7. III. 67	19.2	13.7	85	10	5	0	80	20	0	0	
	22. VII. 67	5.5	2.7	80	20	0	0	100	0	0	0	
IX	10. IV. 67	50.4	41.5	76	24	0	0	-	-	-	-	
	13. X. 67*	81.0	41.5	90	10	0	0	40	10	20	0	
X	5. V. 67	9.2	7.3	74	26	0	0	-	-	-	-	
	5. IX. 67	9.0	8.3	-	-	-	-	-	-	-	-	
	18. X. 67*	35.8	12.8	45	20	5	30	15	5	0	80	
XI	25. V. 67	28.4	9.6	60	28	12	0	90	10	0	0	
	14. X. 67*(3)	56.1	16.6	80	15	5	0	60	10	20	0	
	28. X. 67*	44.3	26.6	20	50	0	30	60	0	0	40	

(1) Thousands per gram. (2) Containing yeasts (30%) (3) Containing cladosporium herbarum (10%)

* Harvested in September 1967.

amount; "*A. candidus*", "*A. glaucus*", "*A. terreus*" and "*A. ochraceus*" existed in minimum amounts or only occasionally. Among penicillii, "*P. italicum*", "*P. expansum*" and "*P. digitatum*" were detected, the former existing in higher proportion. Other genera such as *Fusarium*, *Absidia*, *Alternaria*, *Rhizopus*, *Cladosporium* and *Mucor* were identified also. Out of all molds, *Aspergillii* were the ones predominant; they represented in many samples the major part of molds, and particularly in rices analyzed some months after harvest.

It can be concluded, therefore, that the type and proportion of microorganisms found in commercial samples of freshly milled rice is quite similar to that found in samples prepared at the laboratory and used in previous storage studies.

III. BASIC STUDIES ON RICE QUALITY.

INTRODUCTION.

A major objective of agricultural research of last years has been the development of physical and/or chemical tests to differentiate varieties and lots of rice, evaluate their quality and predict their behaviour on cooking and processing.

Consumer preferences for rice vary widely throughout the world, even within a limited area. In Japan, consumers prefer soft, pasty in texture, sticky rice (53)(325). In Korea (53)(14), Taiwan and parts of China rice with cohesive properties is preferred also (325)(14)(326). In Malaya preference is for flaky and high volume cooked kernels (327). As reported by Juliano (328), "the same criteria for rice quality may hold for Ceylon, Pakistan, South Vietnam, and parts of Thailand". Nevertheless, glutinous varieties are grown in North and North East regions of Thailand for local consumption (384). Glutinous rice also is widely used in desserts in the Far East, especially in China (325). In the Philippines the degree of expansion is also a factor of good quality (329). In this country, as well as in Indonesia, "most persons prefer a rice that remains soft after cooking, even when cold" (328). In India, the rice which absorbs a great amount of water and swells to the maximum extent without becoming slushy, but retaining the fluffiness and the grains remaining well separated is preferred (335). In U.S.A., most consumers prefer rice that cooks dry and fluffy, with a minimum of stickiness and splitting (331). There is however a market for other rice types; for instance, in Louisiana two types are marketed: a) long grain rice and b) medium grain rice which requires less water for cooking and cooks more moist than the former, its grains tend to cling together and can be reheated more successfully (332). In Europe -Italy (333), France (334), Spain (309)- rices giving undisrupted and non-sticky cooked kernels are generally preferred. Nevertheless, for preparing some special dishes, rices giving less separated and less consistent cooked kernels are desired. In Bulgaria, cooking quality requirements are firmness and high volume expansion (349).

In addition to the cited characteristics, others such as grain type, translucency, aroma, are factors influencing consumer's preference. However, the relative importance of the various quality characteristics of rice varies widely from one to another consumer. All that makes necessary -and, in turn, very difficult- to establish an adequate and well specified definition of what it is understood by the term "quality". This however, is lacking in most of the existing information.

Present rice situation demands a selection of varieties. And this need will probably become more acute in a near future -when world rice production will be sufficient (336). Consumers wish to market the type of rice they prefer. Processors also need information on the rice to be used for obtaining a final product of constant and determined quality (337)(338)(339)(340)(341)(305)(306)(342)(343). Rice breeders need to dispose of simple procedures (requiring small amount of sample) for the selection of new varieties covering specific needs (307)(304)(343)(344). Finally, reliable identification and evaluation of samples based on objective criteria are needed for marketing.

Regulations and standards at the rice market (345)(346)(14)(347)(348) do not cover these needs. The Spanish regulation (348), as well as others in general, states that rice (whether rough rice or milled rice) must be classified in "types" according to variety. Thereinafter, every lot of rice is evaluated according to: a) rough rice: odor, moisture content, foreign matter, admixtures, and insect infested kernels, and b) milled rice: red, "green" (unripened), chalky, damaged and stained kernels. Milling yield and percentage of broken are important factors in final evaluation. Moreover, milled rice is classified as "Granza", "Seleto" and "Primera", standards for these classes differing in the limits for head rice, broken, kernels with defects and impurities.

Evaluation of rice according to the latter criteria is simple. However, the preliminar classification, in "types" according to variety, being essential, presents serious problems. For years, this classification has been based on the knowledge obtained through continued use of varieties, that is to say, quality has been sanctioned by practice. However, although every variety is generally associated with specific characteristics, the particular circumstances of cultivation, harvesting, drying, storage and/or processing may bring about remarkable deviations from the expected eating, cooking and processing qualities. The need for evaluating new rice varieties, of which no properties are known, is another problem. On the other hand, although the variety of a given sample of rough rice can be identified with relative certainty, sure identification of a given sample of milled rice is a difficult task even for rice inspection experts.

The need for a reliable and practical method for determining rice quality, applicable to a great number of samples and to a small amount of rice, has brought about much research. A summary of proposed tests is given in the following section.

LITERATURE REVIEW*

Jones (350) following Warth and Darabsett's work (351), developed a method for evaluating the cooking quality of rice, based on the dispersibility of raw entire kernels in alkali. The procedure has been studied and modified more recently (268)(352). Even though evaluation of alkali effects is subjective, and not always easy, the test is useful in differentiating varieties and has been widely used (304)(307)(302)(353)(354)(386); it is admitted however, that the test is not conclusive (368). Further, there is some evidence that it is not a quality test of general validity (379)(355)(362),

Rao et al. (356) used a procedure, previously described by Rao (357), to determine the water absorption of rice during cooking at near-boiling temperature, and found a close positive correlation between this parameter and quality. That water uptake appears to be associated with quality has also been reported by several workers (295)(333)(359). Water uptake has been widely used in India as a quality indicator, but it has not been found applicable to American varieties (290) nor to Spanish ones (309)(249). Cooking

* "Good quality rice" as used in present report means primarily rice giving on cooking undisturbed and nonsticky kernels, according to criteria given in a previous work(309).

at lower temperatures affords a mean to differentiate varieties (289)(358)(360). According to the former authors, water absorption is related to gelatinization temperature, although there is some degree of relationship between water uptake at low temperature and the cooking and processing characteristics of rice (358), this is little consistent (302). The test proposed by Refai and Ahmad (360), studied later on by De Rege (361), has been reported to be useful to establish rice cooking behavior (354); nevertheless, it is admitted that in order to determine the characteristics of the various varieties of rice, this single method (as others) is not sufficient (355)(354).

Rao et al. (356), working with Indian rice varieties, found a close correlation between amylose content and the quality sanctioned by practice. Later, Halick and Keneaster (290), (based on a procedure developed by Roberts et al. (363) for controlling parboiling), proposed the "starch-iodine-blue test" as a quality indicator of white milled rice. The procedure has recently been modified by Hall and Johnson (364). According to Williams et al. (157), there is a positive correlation between the empirical blue value and amylose content of rice; nevertheless, applicability of such a correlation to estimate amylose content is limited to rices with less than 30% amylose (367). In this connection it is of interest to mention a recent modification to the Lambert - Bouguer - Beer's law for improved precision of amylose results (393)(394)(354). The validity of the "starch-iodine-blue test" has been the subject of much controversy. Along with data supporting its good correlation (or that of amylose content) with quality scores (50)(369)(371)(370), notable exceptions have been published (289)(249)(355)(302)(362). A reasonable explanation has been suggested by Juliano (372)(303): "amylose content is the main factor that affects the gloss, cohesiveness, and tenderness of cooked rice", "but protein becomes a prominent factor for samples of similar amylose contents".

Another quality test based on the starch-iodine reaction - "der Quellungsgrad des Reis" - has been reported by Pelshenke and Hampel (60); this, however, has been scarcely used. "Kurasawa et al. (371), reported that the starch-iodine blue color of the residual cooking liquids of boiled entire kernels was a more sensitive test than that of Halick and Keneaster (290)".

A test, developed by Nava (362), is based both on amylose and amylopectin starch fractions. "Using a 1 g sample, this test makes use of the action of diluted (0.33 N) sodium hydroxide solution, that gives a fairly good separation of amylose and amylopectin fractions after 24 hours at 30°C in a constant temperature oven. After separation of fractions, the amylopectin one is heated to 80°C. during 20 min, prior to the test. The fractions, both at room temperature are precipitated with 1:1 H₂SO₄ sol. and, after 30 min, repose, the relative size of the precipitated particle in the amylose fraction is appreciated. In the amylopectin fraction, the more or less clearness of the supernatant liquid as well as the amount of the precipitate formed, are readed. The quality of any rice varies directly to the size of the particle precipitated in the amylose fraction and the clearness of the supernatant of the amylopectin fraction, but inversely to the amount of the precipitate in the latter. In this way it is easy to select any type of rice, not only for cooking but for industrial uses. as well".

The "gelatinization temperature"^(*) of rice flours has been found to be related to cooking behaviour of rice -water uptake- (289). A positive correlation between gelatinization temperature and cohesiveness has also been reported (302). Nevertheless, the later authors reported: "No single characteristic of rice included in this research" (gelatinization temperature among others) "was related to more than 50 percent of the variation in cohesiveness scores. About one-third of the variation in cohesiveness scores of cooked rice was associated with the gelatinization temperature". Other workers have found a lack of correlation between gelatinization temperature and eating quality (303)(309). In this connection, the following comments, reported by Juliano et al. (303), are of interest: "The reported correlation between gelatinization temperature (evidenced by alkali-test values) and the eating quality of rice, particularly stickiness, may be due primarily to correlation with amylose content. A high positive correlation between gelatinization temperature and amylose content (reflected by iodine-blue color) had been reported by Tani and Kubo (373) and Refai and Ahmad (374). This inconsistent correlation between gelatinization temperature and amylose content has not been fully appreciated and has unfortunately resulted in extensive use by many investigators of gelatinization temperature -rather than amylose content- as the eating quality index". Anyway, the gelatinization temperature seems to be useful in differentiating varieties and is being used in the evaluation of rice for various purposes (304)(305)(306)(307)(342).

Several other tests to differentiate rice varieties and/or evaluate their quality characteristics have been developed. Out of them the following can be mentioned (**): the amylogram, which gives the pasting behaviour of rice (289); the oryzogram, which measures consistency of swollen rice kernels (365); the measurement of viscosity of swollen rice kernels with the Haake's consistometer (366); the plastogram, which informs on rheological properties of gelatinized cereals without changing their shape (375); the soaking test, which shows the resistance to disintegration of cooked kernels soaked in distilled water overnight (290); the differential reaction of milled rice to a Millon reagent (376), reported to be related to palatability characteristics; and the differential response of rice starch granules to heating in water at 62°C (377), reported to be correlated with panel scores for cohesiveness of cooked rices.

As it can be seen above, the best part of the tests developed are based on the major constituent of rice, its constituents, or characteristics and properties related to them. Investigations at the author's laboratory directed to develop a quality test for rice followed a different approach. Quality studies were primarily focused in the role of the protein material in the cooking behaviour and eating quality of rice.

(*) "At the concentration (50 g rice flour and 450 ml of water) of rice slurry used" by the authors (289) "to obtain the normal amylograph curve, the initial viscosity increase of the rice pastes was ill-defined and covered a range of several degrees". Increasing the rice-to-water ratio (100 g rice flour and 400 ml. water), the viscosity rise abruptly, and "gelatinization temperature" agrees well with gelatinization temperature by conventional methods (308).

(**) Although not intended for evaluation of eating qualities, but in connection with quality tests, it might be of interest to mention the hard centers test to determine cooking time (378)(381)(384)(354)(355).

Protein content of milled rice varies^(*) from about 4.5% to 14.3% (see Table I, pg. 5). It was found in preliminary investigations, that rices with higher content usually were of better cooking quality; however, exceptions were observed in later studies (172) (125). Investigation of the outer layer of the cooked (382)(172) as well as of the raw (see pg. 11) milled rice kernel showed that the chemical composition of outermost layer differs greatly from that of the entire kernel; proteins occur in it at a very high level (20% or more, according to variety); it suggested a role for this component more important than that previously thought. During cooking, rice undergoes swelling -due primarily to starch-, bringing about rupture of cell walls, particularly in the outermost layer of the kernel (133). Part of swollen starch granules, gelatinised and partly disrupted, scapes and losses starchy material into the cooking water increasing its viscosity, and part remains on the cooked kernel producing a sticky surface. It seemed highly probable that proteins in outer layer tended to coagulate and blocked up starch granules during cooking, affecting the rheological characteristics of the cooked kernel. Subsequent investigations showed that rices with higher protein content in outer layer gave after cooking more entire and less sticky kernels (172)(75)(125). On the basis of this relationship, a procedure to estimate the cooking and eating qualities of rice was developed (74). The test consists in extracting entire milled rice kernels with a biuret reagent, and evaluating colorimetrically proteins in the extract. Results are expressed as an empirical "N index". Differences in grain size and shape influence results, and a correction factor was introduced (173). (More data on N index and quality are reported later on).

Rice quality investigators in the Philippines (369)(372)(303)(383) have also studied the relation of protein content to cooking and eating qualities of milled rice. Protein content and palatability characteristics (tenderness, cohesiveness) were found to be correlated. However as it has been mentioned elsewhere (see pg. 132), in opinion of these researchers, "amylose content is the main factor that affects the gloss, cohesiveness and tenderness of cooked rice. Protein is a recondary factor affecting the texture of cooked rice, but this becomes a prominent factor only for samples of similar amylose contents". These observations were based on data for the average protein content of the entire kernel; no data for the protein content of outer layer were reported.

PLAN OF WORK

As stated previously (see pg. 1) a main purpose of present research project was to develop basic knowledge of the factors determining the quality of rice. As it has been seen in the Literature Review, research on this problem is needed. There is no quality test which has been thoroughly proved to be of universal validity for its ultimate purpose, and, at the same time, there is evidence for the interaction of various factors on rice behaviour, the knowledge of which is far from complete.

(*) In an extensive work carried out at the IRRI, the protein content of brown rice for 4,023 samples from the world collection ranged from 5.6 to 18.2 percent wet basis (40).

In the present project, quality investigations were approached in various ways. In the first one, the possible relation of sulfhydryl and disulfide groups contents to eating quality of rice was investigated. As it has been seen in the previous section, there is a relationship between protein material and rice behaviour during cooking. However, total protein content of rice and of outer layer remain practically unchanged (see Part II). Therefore, interest arose in ascertaining whether changes in chemical nature of proteins were factors responsible for storage changes in rice quality. Both the mechanism of action of chemical improvers on wheat flour and the maturation of flour through aging involve -SH and -SS- groups reactions. The occurrence of similar reactions in rice during storage was considered feasible and worthy to be investigated.

Another approach was to study comparatively the concomitant changes during storage in composition, physicochemical properties, and organoleptic characteristics, and to determine the existence or lack of correlation among them. A previous study carried out at this laboratory (249)(250) proved to be useful for discriminating rice quality factors. In this part of the work, data from the study of storage changes in milled rice reported in Part II, will be used.

The above mentioned studies -which will be reported later on - indicated that the existing knowledge on the role of chemical constituents in rice properties is still incomplete. As regards quality, the amount of proteins present in the outer layer is a useful criterion to evaluate rice but it is not always sufficient. Relationship between rice properties and proteins are not sufficiently defined. As regards storage, knowledge of the relationships between compositional and quality changes also is fragmentary. There are noticeable differences in quality between cooked samples of new and aged rices. The surface of the aged kernel is dryer, less smooth and less cohesive; the cooked aged kernel also is more entire, firmer in texture and larger in size. Obviously, such differences are due to the storage changes in the chemical constituents of the kernel. However, in spite of the great deal of information now available on such changes, it is difficult to explain and justify the differences in cooking properties between new and old rices through the differences in their chemical constituents.

Both quality and storage problems present a common feature: the lack of a way, other than the simple mathematical correlation, to relate the physicochemical and organoleptic characteristics of the cooked kernel with the chemical composition of raw rice. In other words, the lack of a way allowing to reason and interpret the properties of the cooked kernel through chemical data. The great many data now available on properties and composition of rice might thus be more useful to find out the mechanism of cooking and the influence of chemical constituents on the properties of the cooked kernel.

In planning further research, attention was paid to histological and histochemical studies as a mean to establishing the needed connection among chemical, physicochemical and organoleptic data. Knowledge on the microscopical structure of the cooked kernel appeared to be promising in this respect.

On the other hand, the evidence for important changes in nitrogen compounds during storage, and the undoubted relationship between proteins and cooking properties

of rice, moved us to study more thoroughly the modification of the protein material during aging of rice. The study was intended to obtain information on the mechanism(s) of protein alteration and to find out the characteristics of the protein material influencing the properties of aged rice.

Data obtained on storage changes in protein solubility, alpha-amino N and susceptibility to trypsin attack, as well as on formation of free carbonyl compounds suggested the possibility for an interaction of proteins with breakdown products of lipid oxidation. The study of the occurrence, or not, of such a reaction was undertaken.

In summary, the plan of work of present quality studies included the following chapters:

I. Studies on the relation of sulfhydryl and disulfide groups to eating quality of rice: 1. SH and SS contents. 2. SH and SS indices.

II. Application of storage changes data to discriminating quality factors.

III. Study of the microscopical structure of the cooked rice kernel and of its relation to proteins, starch and organoleptic characteristics.

IV. Studies on the occurrence of an interaction of proteins with breakdown products of lipid oxidation during storage of milled rice.

EXPERIMENTAL

I. STUDIES ON THE RELATION OF SULFHYDRYL AND DISULFIDE GROUPS CONTENT TO EATING QUALITY OF RICE.

Methods were described in Part I (pgs. 20-23) and II (pgs. 71-74).

II. APPLICATION OF STORAGE CHANGES DATA TO DISCRIMINATING QUALITY FACTORS.

Data obtained in Part II were used in this study.

III. STUDY OF THE MICROSCOPICAL STRUCTURE OF THE COOKED RICE KERNEL AND OF ITS RELATION TO PROTEINS, STARCH, AND ORGANOLEPTIC CHARACTERISTICS.

Materials

Rice samples of Balilla x Sollana variety, from 1967 and 1968 crops, were used in histochemical work. Bomba and Francés rice varieties, 1968 crop, all grown in Sueca, Valencia, were used when studying the influence of the geometry of the husked kernel on the properties of milled rice. To study the effects of certain enzymes on microscopical structure and properties of cooked rice, rice samples of Balilla x Sollana variety, 1967 crop, were used. To study the effects of aging on microscopical structure and action of certain enzymes on rice, rice samples of Balilla x Sollana variety, 1967, crop, were used. Samples with 14.0% moisture content and 8% milling degree were packed in air-

tight glass bottles and stored at $\pm 5^{\circ}\text{C}$ during two months -"new rice"- and at $\pm 35^{\circ}\text{C}$ during five months -"old rice"-. Milling was carried out at an experimental mill, using rice husked in a laboratory rubber-disc shelling machine.

Methods

a) Histological and histochemical methods. Samples of milled rice were cooked in boiling water and treated as described by Little (385) for subsequent histochemical study. Microtome cross sections of five and ten microns thick were prepared using a MSE microtome, 9001 model. Sections were stained with erythrosin B (for proteins), ruthenium red (for hemicelluloses), iodine-vapors (for starch), and Sudan IV (for lipid material). Photomicrographs were obtained in a Photomicroscope II, Zeiss.

b) Enzyme-treatments. Entire kernels of raw milled rice (20 g) were digested 8 hrs. at $\pm 37^{\circ}\text{C}$ in water (40 cc) containing trypsin (0.1 g), alpha-amylase (0.1 g) or beta-amylase (0.05 g) according to the case; then, rice was cooked in boiling water.

c) Susceptibility to trypsin attack. 20 g samples entire kernel or rice flour were digested 8 hrs, at 37°C in water (40 cc) containing trypsin (0.1 g); 20% trichloroacetic acid were added and the mixtures filtered; nitrogen was determined in the filtrates using Kjeldahl's method (154). Results are given as g N rendered soluble by trypsin/100 g rice flour dry basis or 100 g N. Alpha-amino N: Five grams samples of milled rice flours were digested 24 hrs at 37°C in Sorensen's buffer (50 cc) containing trypsin (50 mg); 20 ml of 20% trichloroacetic acid were added and the mixtures centrifuged and filtered; alpha-amino N was determined in the filtrates, using Sorensen's method (146).

d) Available lysine. It was determined by the method of Carpenter (391).

IV. STUDIES ON THE OCCURRENCE OF AN INTERACION OF PROTEINS WITH BREAKDOWN PRODUCTS OF LIPID OXIDATION DURING STORAGE OF MILLED RICE.

Materials

Balilla x Sollana rice variety, 1967 and 1968 crops, was used. Dehulling and milling of samples were carried out as described in previous section.

To study the effects of formaldehyde on properties of rice, milled rice was held during twelve hours at room temperature in a formal-saturated atmosphere and then left during three days at room atmosphere.

To study the effects of aging on composition and properties of milled rice, samples with 14.6% moisture content, 6.9% milling degree, were packed in air-tight glass bottles and stored at $\pm 25^{\circ}\text{C}$.

To study the effects of defatting previous storage on composition and properties of aged milled rice, entire kernels of rice (6.9% milling degree) were extracted in a Soxhlet apparatus for 8 hours with ethyl ether; after extraction, solvent was drained off and the rice was left on a wire tray at room temperature until practically free of solvent odor. Samples were packed in air-tight glass bottles and stored at $+25^{\circ}\text{C}$.

To study the effects of defatting previous curing, on composition and properties of cured milled rice, samples of milled rice and of defatted milled rice were cured, at $78 \pm 2^{\circ}\text{C}$, for 18 hours, in hermetically sealed glass bottles.

To study the effects of addition of methyl linoleate on composition and properties of aged defatted milled rice, 8 g of methyl linoleate dissolved in 500 ml ethyl ether was sprayed on 1 Kg of previously defatted milled rice; solvent was left to evaporate. Methyl ester of linoleic acid was prepared using diazomethane, which was prepared as described by Boer and Backer (387). Samples of milled rice with methyl linoleate added were stored under O_2 at $+37^{\circ}\text{C}$, for two months.

Methods

Most of the methods used have been described previously (see pages 20 to 23, 71 to 74, and previous section). Others were as follows: 1. Isolation of starch: It was carried out as described by Wheland (388); defatting was carried out according to Schoch (389). 2. Swelling power and solubility of isolated starch: the procedure of Schoch (390) was followed. 3. Amylogram.- A Brabender amylograph, VSK4 model, Duisburg, Germany, was used. The procedure followed was that described by Mazurs et al., (392). Starch concentration used was 35.0 g starch in 450 cc distilled water.

RESULTS AND DISCUSSION

I. STUDIES ON THE RELATION OF SULFHYDRYL AND DISULFIDE GROUPS CONTENT TO EATING QUALITY OF RICE.

In a preliminar work, sulfhydryl and disulfide contents of white milled samples from nine Spanish rice varieties were determined. The SS groups content was found to be positively correlated with cohesiveness and overall acceptability of cooked rice. Later studies on varietal differences in SH and SS distribution in the rice kernel (see pg. 29) showed that SH and SS contents in the outer layer of a good quality rice -Bomba variety- was much higher than that of a poor quality one -Balilla-. Consequently, investigations were continued to ascertain the possible role played by these groups in the behaviour of rice during cooking.

In this connection it was considered of interest to dispose of a quick method for estimating SH and SS groups in outer layer, in order to determine rapidly existing differences among a great number of varieties. A procedure, described in pg. 73, was

developed and used for this purpose. The SH and SS indices obtained by this method for thirteen rice varieties were compared with panel scores for cohesiveness and overall acceptability of cooked samples (Table LIV). SH index was not correlated with quality; on the contrary, SS index appeared to be positively correlated with it. Nevertheless, subsequent studies based on comparison of compositional and quality changes of milled rice during storage -reported in the following chapter-, failed to confirm this relationship.

II. APPLICATION OF STORAGE CHANGES DATA TO DISCRIMINATING QUALITY FACTORS.

In the storage studies reported in Part II, the full sequence of changes in quality, physical and chemical properties, as well as chemical composition of milled rice was established; the number of studied parameters was extensive and practically all the data obtained were confirmed, at least, in two different experiments. This information allows the study of the existing relationships among the various characteristics and the application of it to discriminating factors involved in quality.

Water absorption and residual solids from cooking (pg. 85) undergo significant changes during storage, which must be taken into account when using stored rice, particularly in technological processes such as canning, precooking, etc. Although their changes appear to be associated with those of quality, these parameters are not useful criteria to measure neither the original quality of rice nor its variations due to storage. Samples with similar values for water absorption or for residual solids exhibited different eating qualities.

Neither the gelatinization temperature (pg. 86) nor the alkali digestibility value (alkali test, pg. 86) are useful criteria to follow the effects of storage on the quality of milled rice. These parameters neither show differences due to quite different holding conditions nor undergo significant changes during storage. As to pasting characteristics, it has been stated elsewhere (pg. 93) that although, in general, a parallel increase of peak viscosity, set-back and quality could be admitted, this relationship did not hold a detailed comparison.

SH and SS indices were not useful to measure storage effects on rice quality. The relationship previously found between SS index and quality did not hold in samples stored under different conditions. SS as well as SH groupings underwent rapid and highly significant changes which did not parallel those in quality (compare Figs. 18 and 10).

Comparison of storage changes in rice properties and rice composition shows that there is not a relationship between the amount of any of the major constituents of rice and the quality. Starch, protein and lipids remain practically constant in quantity whereas rice properties change significantly. Out of the fractions of major constituents investigated only some deterioration products of lipids and some protein solubility fractions appear to be of interest.

Table LIV. - SH and SS indices and other characteristics of rice.

Varieties	Cohesiveness scores	Overall acceptance scores	SH index		SS index		N index		Protein %
			a	b	a	b	a	b	
Bomba	8.0	7.4	124	447	624	2250	246	887	10.62
Quarantatre	7.2	6.1	81	245	615	1862	289	875	12.14
Capataz B	6.8	6.1	108	350	652	2113	257	833	11.19
R. Bersani	6.6	5.9	119	342	715	2053	278	798	11.83
Commercial sample	6.6	6.2	142	447	535	1799	179	602	8.06
Stripe 136	6.3	5.8	110	371	556	1876	242	817	9.79
Liso	6.3	5.6	102	336	548	1804	211	694	8.57
Balilla grueso	6.4	5.3	86	295	502	1723	211	724	9.31
Balilla	6.3	5.7	105	335	531	1694	211	673	8.89
Bluebonnet	6.2	5.1	150	536	379	1354	190	679	7.08
Toro	5.5	4.2	182	704	351	1358	160	619	7.60
Nato	6.3	5.3	115	475	373	1540	129	533	6.74
Glutinous Zenit	1.8	1.7	166	631	317	1205	126	479	6.70

(a) Uncorrected values.

(b) Surface corrected values.

The role of lipids is however limited to storage changes (it will be dealt with later on). Alkali soluble proteins of the outer layer appear to be involved in both original quality and storage changes; nevertheless, exceptions were observed and these are noted below.

The lack of significant changes in amylose content (pg. 98), in contrast with the loss in amylose solubility during storage of rice (249)(259)(402)(225)(224)(272), suggests: a) amylose content appears to be an unreliable index for evaluating stored rices, and b) the correlation between amylose content and the starch-iodine blue value appears to be affected by storage of rice; the possible limitation of the use of the latter for rapid determination of the former in stored rices seems to be worthy of study.

When the effects of storage on a) cohesiveness (pg. 79), b) overall acceptability (pg. 79), c) alkali soluble proteins (pg. 105), and d) N index (pg. 93) are compared, the following trends, associated with storage temperature, are observed: At $+5^{\circ}\text{C}$, cohesiveness and preference remain practically constant. So do the alkali soluble proteins of outer layer; N index remains constant initially but after two-four months increases. At $+25^{\circ}\text{C}$ cohesiveness scores increase slightly -rice becomes lesser sticky. Preference increases too but meanwhile rice does not become deteriorated; when off-flavors appear, preference decreases. Alkali soluble protein does not change significantly at the beginning; on prolonged storage it decreases. N index increases parallelly to cohesiveness and preference; when rice deteriorates, preference decreases at once, N index continues being parallel to cohesiveness until deterioration is strong and then decreases. At $+35^{\circ}\text{C}$ cohesiveness improves and preference decreases. Alkali soluble proteins of outer layer and N index decrease.

Existence of a relationship among the cited characteristics is clear. However when evaluating it, the following is noted: 1) The proportion of alkali soluble proteins in the outer layer is not sensitive enough to show quality changes. 2) In general, there is good correlation between quality and N index changes. Meanwhile rice does not become deteriorated, cohesiveness and preference improves and N index increases. When rice deteriorates, N index tends to decrease, the change depending on the degree of deterioration. As deterioration involves easily detectable off flavors, evaluation of quality has no problem. After prolonged storage at $+5^{\circ}\text{C}$, N index increases whereas quality remains constant. This constitutes a deviation of the correlation between quality and N index which remains to be explained.

A storage experiment carried out with thirteen commercial rice samples stored in the laboratory from February to September at room conditions supplied additional evidence of the correlation between N index and quality. The samples were purchased in different shops, and included rices of different types, commercial qualities and prices. Results for cohesiveness, preference and N index are given in Table LV. From them the following correlation coefficients were calculated:

February: cohesiveness \times N index = 0.692 significant at the 1 per cent level
 preference \times N index = 0.783, id.

September: cohesiveness \times N index = 0.736, id.
 preference \times N index = 0.560, significant at the 5 per cent level

Table LV.- Effects of storage on cohesiveness, preference, and N index of thirteen commercial rice samples.

Samples	Cohesiveness		Preference		N index	
	February	September	February	September	February	September
I	5.8	6.6	5.7	6.1	199	212
II	6.2	6.5	6.1	5.8	229	219
III	6.2	6.2	6.0	5.9	200	202
IV	6.3	6.7	6.5	6.1	221	204
V	6.3	6.4	6.2	5.7	200	214
VI	5.8	6.2	5.8	5.9	213	209
VII	5.8	6.7	5.6	6.0	175	196
VIII	6.3	6.3	6.7	5.9	206	207
IX	8.6	8.3	8.3	8.1	252	252
X	7.5	7.4	7.0	5.7	247	245
XI	7.2	7.3	7.2	6.3	265	252
XII	7.1	7.1	6.5	4.9	198	198
XIII	6.3	6.7	6.3	6.4	218	217

It will be noted that the correlation between N index and preference was lower at the completion of the experiment than at the beginning, whereas that with cohesiveness remained unchanged. It was due to incipient deterioration of some of the samples, which resulted in somewhat lowered preference but slightly increased cohesiveness. The same effects were noted in the storage studies reported in Part. II.

The data obtained in present and previous (74)(75)(172)(173) studies showed the interest of the N index in rice quality evaluation. It was believed nevertheless, that further studies were necessary to know the cause(s) of its changes during storage and to explain and correct its deviation at $\pm 5^{\circ}\text{C}$. Investigation of these problems are reported in the following chapters.

III. STUDY OF THE MICROSCOPICAL STRUCTURE OF THE COOKED RICE KERNEL AND OF ITS RELATION WITH PROTEINS, STARCH AND ORGANOLEPTIC CHARACTERISTICS.

Works investigating the relationships between chemical composition and physicochemical or organoleptic properties of cooked rice through microscopical structure are scarce. The papers by Desikachar and Subrahmanyam (133) and by Little and Dawson (20) are of interest in this connection. The former authors examined under the microscope free hand cross sections of cooked samples of new and old rice "with a view to observing the integrity or intactness of cell walls" and to finding out whether any difference did exist between their cell wall structure after cooking. They certainly found it. Although both the new and the old rice kernels have a surface layer made of disrupted cells due to cooking, such a layer is thicker in the new than in the aged kernel. This difference seems to correspond well with others known to be between both rices, such as integrity of the

kernel, solids dispersed into the cooking water and the tendency to cook to a pasty mass. Parallel studies carried out with parboiled rice showed that cells preserved their original shape and remained structurally intact during cooking. It coincides with the firmer consistency and lesser cohesiveness of the cooked parboiled grain. The fact that aging -as parboiling- results in a structure more resistant to desintegration and, therefore, in a better quality, was ascribed by the authors to a hardening of the cell walls. However, they stated that "the exact nature of these changes needed to be further elucidated".

Little and Dawson (20) also attributed to the cell walls the role of "limiting the expansion of starch during cooking and also determining the position of cleavages in the tissue". Moreover, they obtained some evidence in support of the view that proteins could be included among the rice constituents influencing the expansion and disruption pattern of the kernel during cooking. Nevertheless in opinion of the cited authors, "not only the cell walls (thickness, area, and composition) and the protein (composition, and distribution) but also the fat and mineral content" should be the subject for "more detailed histochemical studies to help to clarify the cooking characteristics of different rice varieties".

On the other hand, it has been seen before that a great deal of chemical information is available supporting that proteins are related to the quality of the cooked rice kernel, although more knowledge on their role in the rice cooking behaviour is needed to establish with appropriate detail the existing relationship.

Therefore, particular attention was paid to the protein material when approaching present study of the microscopical structure of rice and its relation to composition and quality.

The work done, reported below, included the following parts: 1) histochemical study of the cooked rice kernel, 2) study of the effects of certain enzymes on the microscopical structure and properties of the cooked kernel, and 3) study of the effects of storage on a) microscopical structure of the cooked rice kernel and b) action of certain enzymes on composition, microscopical structure and properties of the cooked kernel.

1) Histochemical studies on the cooked rice kernel.

Samples of milled rice of Balilla x Sollana and Bomba varieties, of known cooking quality and composition, were cooked in boiling water and treated as described in the Experimental Part for subsequent histochemical study. Microtome cross sections of five and ten microns thick were prepared, stained with iodine, erythrosin B or ruthenium red and examined under the microscope.

The red stain of proteins with erythrosin, in black at the photomicrographs of Figs. 22 and 23, showed: 1st) that proteins occur in cooked rice as granules -more or less discrete- and as dispersed material, and 2nd) that proteins are not homogeneously distributed within the cooked kernel. Protein granules are distinctly visible around the swollen starch granules and near the cell walls where they occur in high concentration, outlining the cell. Protein density appears to be the highest at the outer layer of the cooked kernel -as it is known (172)(20). The outer layer is, however, heterogeneous

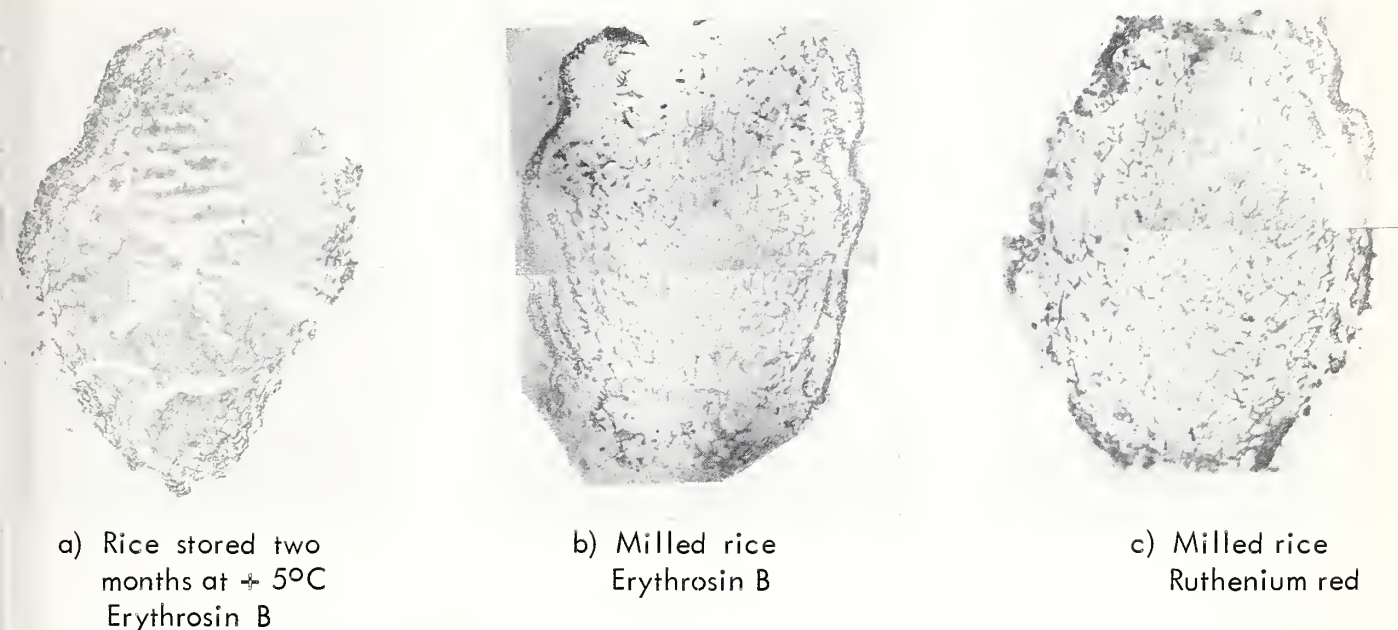
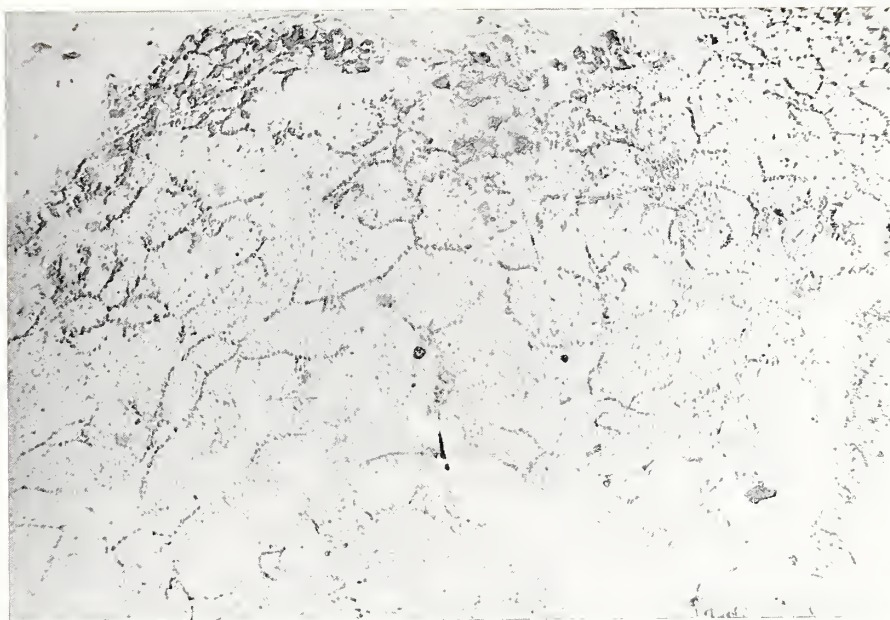


Fig. 22.- Photomicrographs of transections of cooked milled rice. Magnification $\times 17$

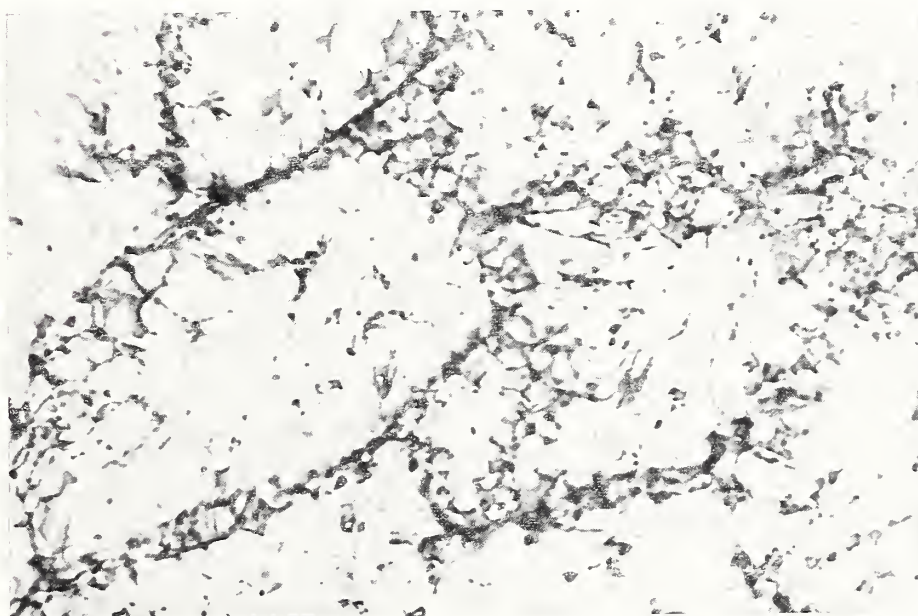
and discontinuous both in composition and cell structure. And it is of interest to point out that composition and intactness of cells appear to be closely related. It can easily be seen in Fig. 22 and especially in Fig. 24, that some areas of the outer layer are very rich in proteins whereas some others are extremely poor. In the former, kernel outline appears well defined and cells, although somewhat disrupted, hold a relatively organised structure, basis of a firm texture. On the contrary, in low protein areas the organised structure has been lost; kernel outline there is not clearly defined, and the outer layer has a paste-like soft texture. Swelling during cooking is easier at these low protein areas where rupture of cells initiates. Examination under the microscope of specimens stained with ruthenium red and zinc-chloride-iodine reagent to show up cell walls, ratified the conclusions withdrawn from erythrosin stainings.

Observations described above were in agreement with those reported previously by Little and Dawson (20), lending support to the view that proteins play an important role in the mechanism of rice cooking. They also were in agreement with the structure expected from histology and histochemistry of the raw kernel (20)(22)(398)(399)(400). Further, the relation between protein content of the outer layer and cooking quality, found previously (172)(75)(74)(173) (see also pg. 141), was thus explained.

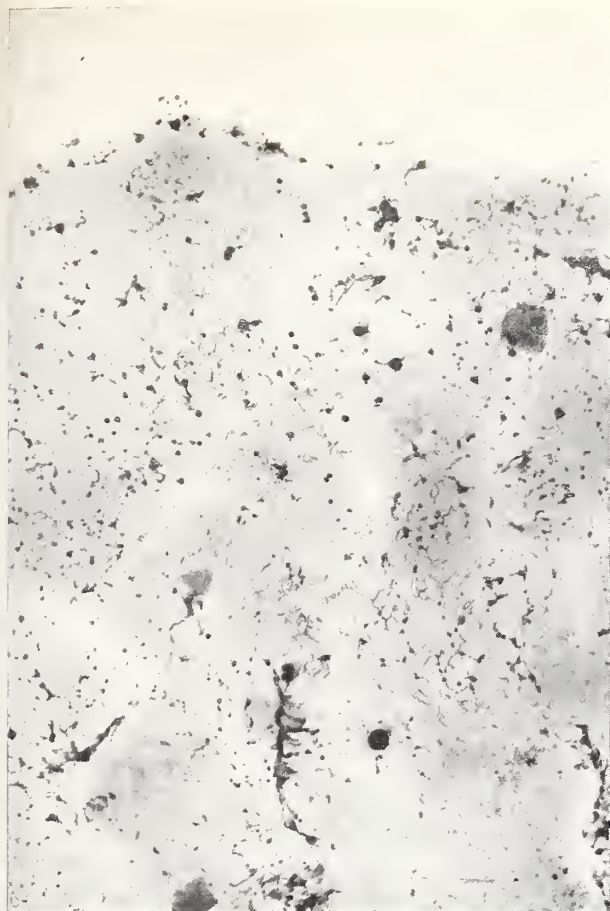
Nevertheless, two questions remained pending: 1st) Whether the relation of proteins to microscopical structure was causal or merely circumstantial. 2nd) Why aged rice kernel, having the same protein content and distribution as new one, is of better quality?. Investigations regarding these problems are reported in subsequent sections. But, before passing to them, it seems worthy to make some further comments on the occurrence of low protein areas within the outer layer.



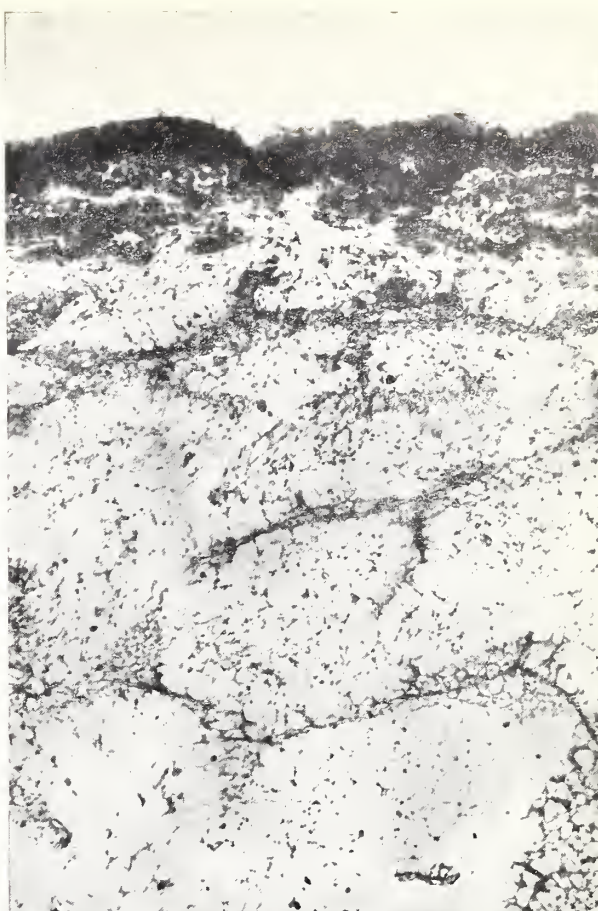
a) Structure of peripheral starchy endosperm.
Magnification: x 60



b) Structure of starchy endosperm.
Magnification: x 510.



a) Ventral-peripheral area, showing cell walls ruptured.



b) Lateral area, undisrupted.

Fig. 24.- Structure of peripheral endosperm of cooked milled rice. Erythrosin B.
Magnification: $\times 235$.

Existence of a continuous high protein outer layer in milled rice kernel depends upon: a) the protein content and distribution within the kernel and b) degree and uniformity of milling. The first condition relates to a characteristic inherent to variety or lot. Therefore only an appropriate selection of rice may be done. The second condition depends primarily on the milling process. Unfortunately, present milling techniques result in non uniform abrasive effects (13)(6). Deep milled areas -generally located along the dorsal and ventral lines- facilitate disruption (as reported by Little and Dawson (20) and commented above) and undermilled areas -the longitudinal creases- bring about distortion and occasionally halving of the kernel. The role of proteins in this behaviour of the kernel has not been sufficiently explored. Therefore, a comparative study of two rice varieties -"Bomba" and

"Frances"-, the caryopsis of which were of quite different cross-section outline (Fig. 25), was undertaken with the purpose of ascertaining the effects of milling on the continuity and density of proteins in outer layer, and the consequences -differentiated from those of true bran- on the properties of the cooked kernel.

As it can be seen in Fig. 26, the abrasive action of the mill removed approximately concentric layers from the Bomba kernel, resulting in a relatively uniform milling. The outer layer of Bomba milled rice was almost homogeneous. A continuous barrier of protein material cemented the outer layer, protecting the kernel from bursting or disintegrating. On the contrary, the Frances rice variety underwent non uniform milling; protein rich subaleurone layers were lost in a major part, the cells there became disrupted and the surface sticky. In contrast with this, bran-free protein rich surface areas -which remained due to undermilling- caused distortion of the kernel. Photomicrograph c) shows the Frances kernel, practically divested of protein rich outer layer by deepmilling; cell structure was lost, surface was sticky, but swelling took place freely without located distortion.

The influence of proteins in kernel behaviour during cooking was therefore substantiated. On the other hand, it was evident that as long as present processing techniques be in use, the geometry of the rice kernel is a factor more important than what actually is generally considered for selection of rice varieties and evaluation of milling quality. In this sense, spherical or cylindrical kernels are obviously the most idoneous for uniform milling and, consequently, for obtaining a milled rice of the best possible cooking quality.

2) Effects of certain enzymes on microscopical structure and properties of cooked rice.

Elucidation of the chemical constituents influencing rice cooking behavior is beset with the difficulty of that it is not possible to compare rice varieties or samples differing in only one component. Several variables occur always making difficult to discriminate individual influences. To overcome this inconvenient, attempts were made to prepare artificially the desired rice samples. Utilization of enzymes was considered appropriate for this purpose since their action on the substrate is specific.

Entire kernels of raw milled rices (20 g) were digested 8 hrs. at 37°C in water (40 cc) containing trypsin (0.1 g) alpha-amylase (0.1 g) or beta-amylase (0.05 g) according to the case; then, rice was cooked in boiling water. Microtome cross sections of the cooked treated samples and their controls were prepared and specimens, adequately stained, were examined under the microscope with a view to finding out possible relationships among cell structure, kernel integrity and composition. The cooked samples also were evaluated organoleptically and results related with previous data.

Beta-amylase treated rice (Figs. 27b and 28d) was similar to control. An outer layer of high protein content outlined the kernel; some areas of low protein content were visible as well as their effects on kernel integrity; presence of residual bran in some points and their influence on kernel outline too. In general, cell structure was relatively well organized. Appearance to the naked eye (Fig. 28d) and texture of the cooked rice were as expected from microscopical structure. Alpha-amylase treated rice (Fig. 28c) was different

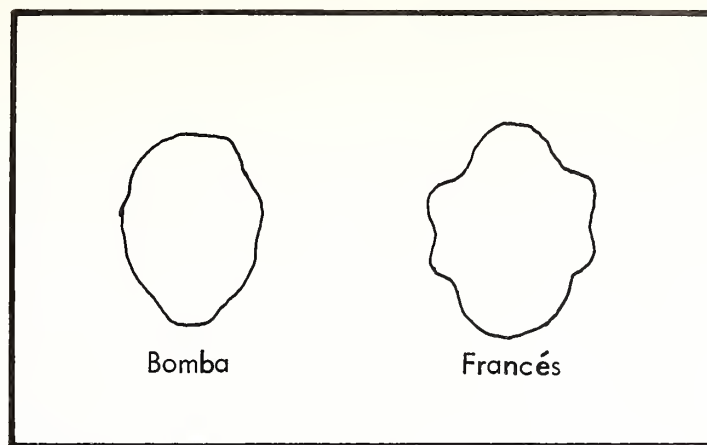
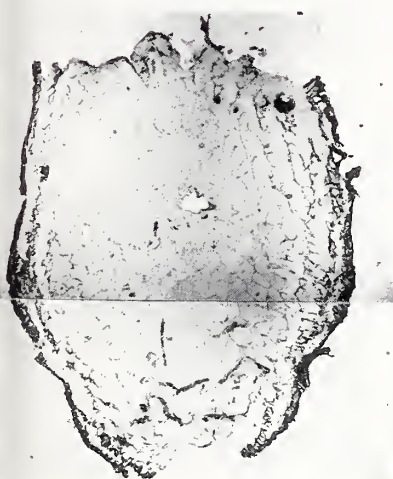
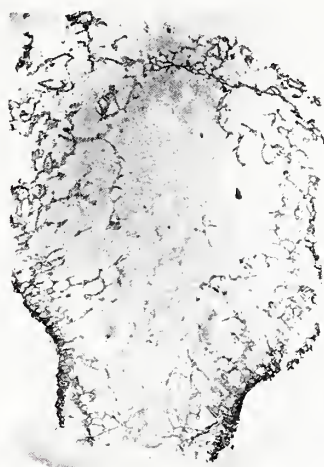


Fig. 25.- Outline of cross-section of raw rice kernels: Bomba and Francés varieties.



a) Bomba
5% milling degree



b) Francés
5% milling degree



c) Francés
20% milling degree

Fig. 26.- Photomicrographs of transections of cooked rice showing the influence of geometry of caryopsis on milling effects upon outer layer proteins and structure of the cooked kernel. Erythrosin B. Magnification $\times 17$.

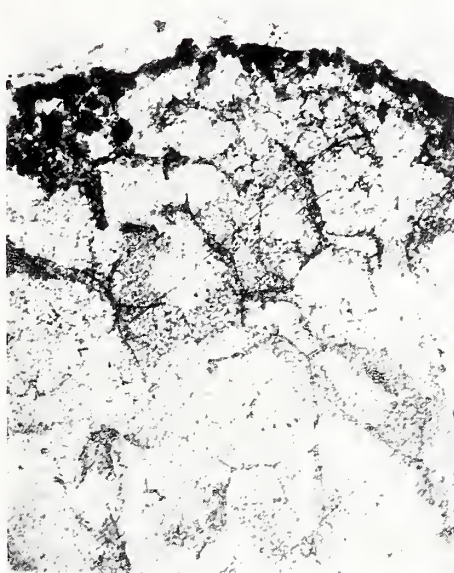
from its control. The cooked kernel was smaller, harder in texture and somewhat gummy. Its cell structure was almost intact—apparently more than that of its control. Cooked kernels had no tendency to stick each other. Trypsin treated rice (Figs. 27 and 28b) also was different from its control, although in another sense. As it can be seen in the photomicrograph of Fig. 27, most of the protein in outer layer was lost. In a major part of the periphery only



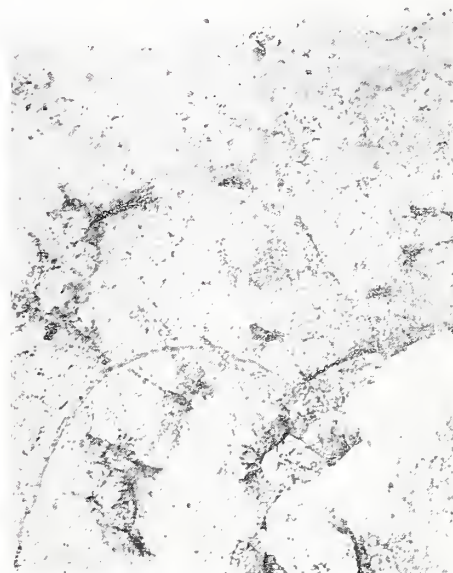
a) Trypsin treated rice (x 20)



b) Beta-amylase treated rice (x 20)



b) Perypheral area of cooked untreated rice (x 160)



d) Perypheral area of cooked trypsin-treated rice (x 160)

Fig. 27.- Photomicrographs showing the effects of enzyme-treatment of raw rice on microscopical structure of the cooked rice. (a) and (b) stained with Erythrosin B; c) and d) stained with Ruthenium red).

the dispersed protein material could be seen. The surface of the kernel was practically a starchy gruel. Cells were disrupted, as shown in Fig. 27 and confirmed through microscopic examination of ruthenium red stained specimens. As a consequence, trypsin treated rice was highly cohesive (Fig. 28b).

The following conclusions can be withdrawn from the observations described above:

a) The predominant role of the outer layer in rice cooking behavior is confirmed. b) The influence of the proteins of the outer layer in the mechanism of cooking is confirmed: Starch absorbs water, increases its volume and viscosity, gelatinises, and tends to disperse. Starch in the outer layer, if free of protein, swells freely and brings about cell disruption, formation of a pasty gruel and, consequently, high stickiness. Proteins surround starch granules and cell walls and, although disperse somewhat, coagulate in a major part. Proteins in the outer layer control starch action, preserve integrity of cell structure, and minimize the tendency of rice to become sticky.

Studies with enzyme treated rices support the view that relationships between proteins in outer layer and kernel integrity or organoleptic properties are causal rather than circumstantial. On the other hand, on the basis of such information it seems improbable that quantity or amylose/amylopectin ratio of starch may counterbalance sufficiently the defects arising from a lack of proteins in outer layer. It seems logical, however, that rices similar in protein material, may differ from each other due to differences in composition or properties of starch. A different point of view on the relative importance of members of the starch-protein binominal has been reported (372).

Effects of enzyme digestion of rice on properties of the cooked kernel suggest some possibilities of enzyme treatment for industrial uses. Application of amylolytic enzyme preparations in canning cooked rice might perhaps be an instance. The amylolytic action on the cooked kernel surface would minimize cohesiveness, avoiding the rice to become a pasty mass during canning and retorting, as well as during storage. Hot water washing previous canning or sterilization would inactivate the enzyme. Amylolytic enzymes could similarly be assayed in processing dehydrated rice. On the other hand, treatment of rice with proteolytic enzymes or addition of these to dried processed rices would make the rice to become somewhat more cohesive during final preparation; as sticky rice is preferred by some sectors of rice consumption (see pg. 130), application of proteolytic enzymes could perhaps open new markets for naturally non sticky rice varieties.

3) Storage effects on: a) microscopical structure of the cooked rice kernel and b) action on certain enzymes on rice.

We have seen in previous sections that proteins in outer layer, cell structure and quality are related characteristics. On the other hand, it is known that storage under adequate conditions may bring about improvement of rice quality. Attempts made to ascertain whether the above cited relationship holds during storage are reported below.

The work included: a) comparative study of the histology and histochemistry of new and aged rices -cooked kernels-, in which particular attention was paid to proteins, and b)

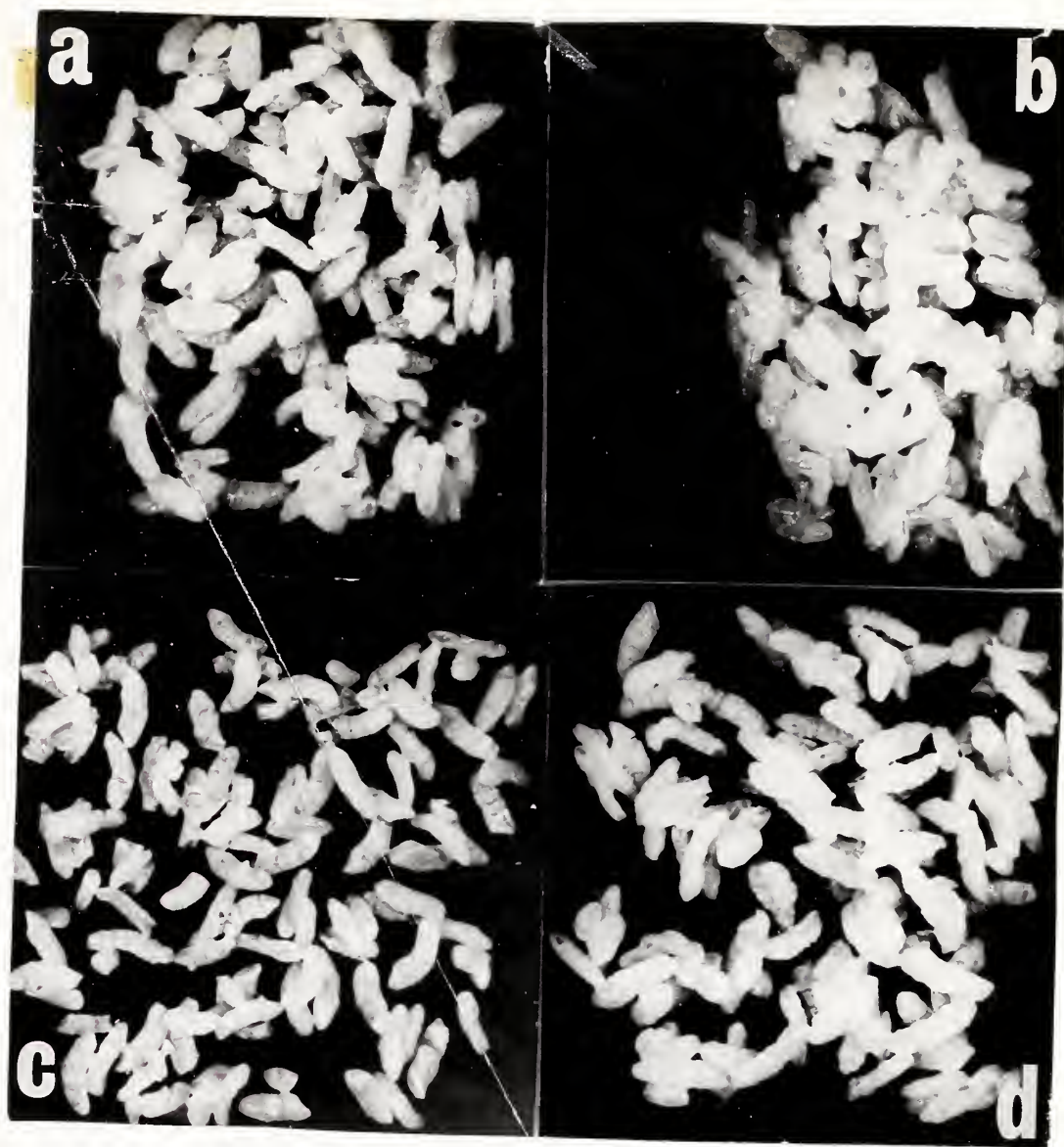


Fig. 28.- Photographs showing the effects of trypsin (b), alpha-amylase (c) and beta-amylase (d) treatments of raw rice on the appearance of cooked kernels. ((a): untreated sample).

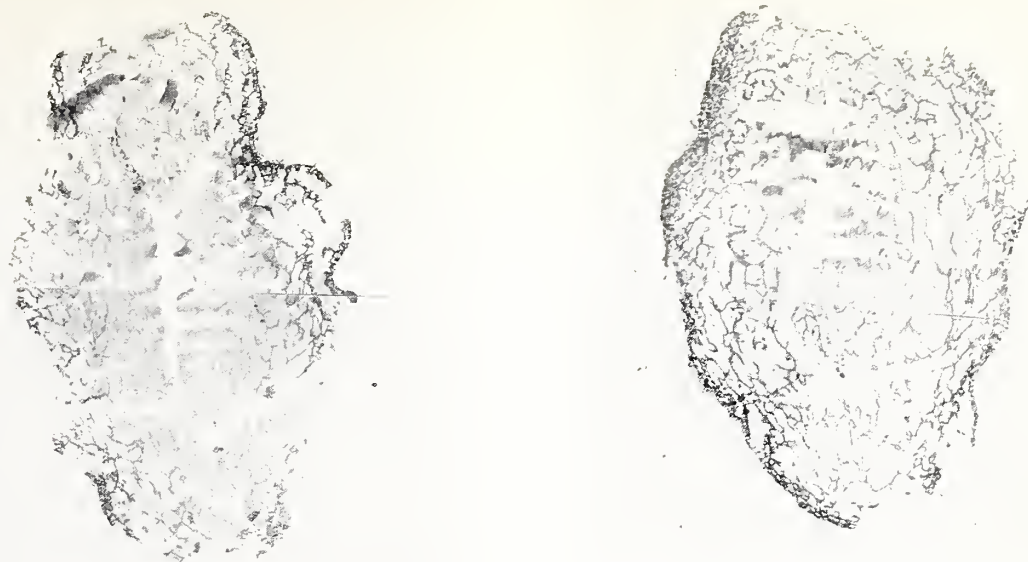
study of the effects of trypsin treatment of new and old rices on organoleptic properties, and histology and histochemistry of cooked kernels. In addition, chemical evaluation of susceptibility of raw new and old rices to trypsin attack was carried out. These studies were also intended to supply information on the causes of rice quality improvement, with particular reference to the participation of proteins. And in this respect, both quantity and "quality" of the protein material were investigated.

3 a) Histology and histochemistry of cooked new and old rices. Samples of the Balilla x Sollana rice variety, were stored as milled rice in air-tight containers at 5°C during two months -sample taken as "new rice"- and at 35°C during five months "old rice". Microtome cross sections of the cooked samples were prepared as usual, and stained with Erythrosin B, iodine vapors, and Sudan IV to study proteins, starch and lipids, respectively. Out of the three constituents investigated, only proteins showed differences between new and old rices (Fig. 29). They were: 1st) The staining of aged rice proteins was deeper. In this connection, a recent paper of Waggle et al (397) is of interest. Steam-conditioning of wheat results in increased staining of their proteins with several stains; dispersibility of the protein decreases parallelly. Whether modification of the protein material is or not in this case -as in curing and parboiling of rice- similar to that due to storage of milled rice is unknown. However it is interesting to point out that all these treatments confer higher integrity and lesser cohesiveness to the kernel after cooking than the original grain. 2nd) Cell structure was more entire in aged than in new rice, corresponding with the better kernel integrity of the former. In agreement with Desikachar and Subrahmanyam (133), this difference was larger in the outer layer than in the nucleus. Saito (353) also noted that the degree of the breakdown of tissues by boiling decreased during storage. 3rd) Strictly speaking, the histochemical evidence so far obtained was insufficient to prove that proteins were responsible for such a difference in structure although it strongly indicated that this was the case. The fact that a more entire structure during aging was concomitant with deeper staining of both protein granules and dispersed material, involving better defined boundaries, suggested it. On the other hand, the influence of proteins proved in previous sections support this interpretation.

3 b) Action of trypsin on cooked new and old rices. Entire kernels of raw milled rice (20 g) were digested 8 hrs. at 37°C in distilled water (40 cc) containing trypsin (0.1 g). After draining the digestion liquid, samples were cooked in boiling water. Parallelly, controls were prepared. Rices used were of the Balilla x Sollana variety, stored as milled rice in air-tight containers at 5°C during two months -taken as "new rice"- and at 35°C during five months -"old rice". Organoleptic analysis and histochemical studies were carried out on prepared samples and controls, and chemical evaluation of trypsin attack was made in digested rices previous cooking.

Organoleptic characteristics. As expected, new and aged rice controls showed different cohesiveness. Old rice was less sticky; it also was more entire. Trypsin treated samples were much more cohesive than their controls -as seen previously-, and old rice was less sticky than new one; it could not be defined whether this difference was equivalent to that between the untreated samples. However, it was proved that when increasing trypsin attack on old rice, the difference between old and new samples disappeared. Results lent therefore further support to the view of the occurrence of changes in protein nature -which presumably strengthen the cementing action of the protein matrix. On the other hand, the fact that new and old rices became comparable in cohesiveness by modifying only the proteins -by trypsin digestion-, indicated that this constituent plays a role in rice improvement through aging.

Histochemistry. Specimens stained for protein and hemicelluloses did not show differences between old and new rices.



a) Milled rice, stored four months
at $+5^{\circ}\text{C}$

b) Milled rice, stored four months
at $+35^{\circ}\text{C}$

Fig. 29.- Photomicrographs of transections of cooked new and old milled rice. Stained with Erythrosin B. Magnification $\times 20$.

Susceptibility of rices to trypsin attack. N rendered soluble and alpha-amino N liberated by trypsin attack (Table LV!) decreased during storage. Changes were larger at $+35^{\circ}$ than at $+5^{\circ}\text{C}$. The outer layer was more susceptible to trypsin action than the nucleus.

Rao et al (92) reported results showing that N rendered soluble by digestion with pancreatin of raw husked, raw undermilled and raw milled rices decreased during 12 months of storage in gunny bags at room conditions. Our data, although not directly comparable, were in agreement with these of the Indian workers. As to the different digestibility of outer and inner layers found by us, it may be said that Rao et al (92) reported a greater solubilization of proteins in husked than in milled rice. However, neither the cited authors, nor Basu and Mukherjee (395) found differences between rices of various milling degrees.

The decrease in trypsin digestibility of rice added further indication of a modification of the nature of proteins during storage. And it is of interest to point out that both cooking quality and susceptibility remained practically constant in rice stored at $+5^{\circ}\text{C}$, whereas they changed in rice stored at $+35^{\circ}\text{C}$. As it is known, trypsin action is quite narrowly restricted to "basic" bonds that is to say, to such bonds which link the carboxyl group of a basic amino acid -arginine and lysine- to the amino group of another amino acid or to the hydroxyl group of an alcohol; when the epsilon- NH_2 groups of the lysine residues are blocked, trypsin splits only arginyl bonds (396). Accordingly, the obtained data might be interpreted as an indication of loss of free epsilon-amino groups of lysine during storage. In fact, a significant loss in available lysine was found after one year's storage of 9.8% milled rice, with 13.0% M.C.,

Table LVI.- Effects of storage on susceptibility of raw milled rice to trypsin attack.

Samples	Storage at + 5°C		Storage at + 35°C	
	New rice	Aged rice(1)	New rice	Aged rice (1)
<u>Solubilised N (2)</u>				
Entire kernel (3)	0.11	0.11	0.11	0.07
Entire kernel flour {	(3) 0.41	0.41	0.22	0.14
	(4) 23.8	23.8	13.0	8.3
Outer layer flour(5) {	(3) 1.11	1.09	0.83	0.51
	(4) 38.4	32.7	28.7	16.8
<u>Alpha-amino N (6)</u>				
Entire kernel	-	-	188	131
Outer layer flour(5)	-	-	326	146

(1) Four months before solubilised N determinations; five months before alpha-amino analysis.
 (2) N rendered soluble by trypsin attack. See Experimental. (3) g N rendered soluble by trypsin/100 g rice or rice flour, dry basis. (4) g N rendered soluble by trypsin/100 g N. (5) 5% of the kernel weight. (6) mg alpha-amino N/100 g rice flour, dry basis.

at + 35°C. Available lysine decreased from 3.05 to 2.61 g/100 g protein. Ben-Gera and Zimmermann (401) have also reported that lysine with a free epsilon amino group decreased during storage of various proteinaceous foods (rice was included in the study). It agrees fairly well with the possibility, commented elsewhere, of an interaction of proteins with breakdown products of lipid oxidation. Investigations reported in the subsequent section deal with this problem.

IV. STUDIES ON THE OCCURRENCE OF AN INTERACTION OF PROTEINS WITH BREAKDOWN PRODUCTS OF LIPID OXIDATION DURING STORAGE OF MILLED RICE.

Available data on the effects of storage of milled rice on kernel constituents had shown that proteins undergo changes in their chemical nature. Protein remains practically constant in quantity but storage results in loss of solubility, increased resistance to trypsin attack and deeper staining with Erythrosin B. On the other hand, some evidence was obtained supporting the view that such modifications are related to kernel behavior on cooking. Due to its basic interest both for storage and quality problems, work was initiated to obtain more information about it. In this connection, the possibility of an interaction of proteins with breakdown products of lipid oxidation, as one of the reactions involved, was considered feasible. There were several reasons for it: a) Improvement of rice quality during storage proceeds under conditions favouring deterioration of lipids. At + 25°C both proteins and quality change. At

+ 5°C both parameters remain practically unchanged. b) It has been reported (299) that acetaldehyde, propionaldehyde or acetone, methyl ethylketone, n-valeraldehyde and n-caproaldehyde are formed in rice during storage, presumably arising during autoxidation of lipids. These compounds are potential protein cross-linkers. c) As expected from changes in peroxide and iodine values of lipids, free carbonyl groups contents of rice should reasonably increase significantly during storage. However, this is not the case; thereby it may be reasoned that free CO are combined at least in part, as formed.

In this connection, a paper by Desikachar and Subrahmanyam, published in 1960 (101) is of special interest. The cited authors reported: "Prior soaking of the new rice in 10% formalin overnight (16 hours)" at laboratory temperature (25°-26°C), "enhanced the swelling quality of new rice to the same level as that of old rice... Also, the cooked grains did not stick to one another". "Formalin is known to bring about crosslinking across starch molecules and also to combine with proteins and is used for this reason as a structural fixative agent. The fact that formalin improved the cooking quality of the new rice therefore indicates that the better cooking quality of the old rice could be due to its hardening during storage". Treatment of rice with formalin, as carried out by the Indian workers, seems to favour hemiacetal rather than acetal formation with starch. Formation of cross-linked proteins seems more feasible. In any case, meanwhile chemical evidence be not presented, conclusions can not be withdrawn.

Studies were therefore initiated to obtain experimental data indicating whether a protein-carbonyl interaction takes place in rice during storage. In this connection, storage changes in rice and the effects of treating new rice with carbonyl compounds were comparatively studied; they should present parallel trends.

In addition, to obtain data regarding the role of lipids in protein changes during -normal or accelerated- storage, the following comparative studies were planned to be done: a) Effects of defatting previous storage on composition and properties of aged milled rice. b) Effects of defatting previous curing on composition and properties of cured milled rice. c) Effects of addition of methyl linoleate and subsequent storage on composition and properties of defatted milled rice.

1. Effects of carbonyl compounds on composition and properties of milled rice.

In a preliminary stage, samples of milled rice were treated with various carbonyl compounds -formaldehyde, acetaldehyde, heptaldehyde, cinnamaldehyde, and furfural- under different conditions; the treated rices were cooked in boiling water and evaluated organoleptically in order to establish the more appropriate treatment. The rice held during twelve hours at room temperature in a formal - saturated atmosphere and aerated during three days was the sample selected. The procedure is simple, avoid soaking of rice and brings about significant changes in the cooking behavior of the kernel. Treated milled rice and its control were characterised through the parameters listed below, of which storage changes are known. A) Organoleptic characteristics: 1) Cohesiveness, 2) color, 3) integrity. B) Physical and chemical characteristics: 1) size of the cooked kernel, 2) residual solids from cooking, 3) water absorption, 4) N index. C) Composition: 1) total N, 2) total alkali soluble proteins, 3) alpha-amino N liberated after trypsin hydrolysis, 4) amylose content. D) Effects of trypsin attack: 1) cohesiveness of kernels cooked after enzyme action, and 2) susceptibility to trypsin attack.

Table LVII reports the results obtained; it also includes the pattern of changes for every characteristic during storage of milled rice. Examination of table's content reveals that formol treatment and storage have quite similar effects on rice. One very minor exception is observed -water absorption- and this is noted below. It is interesting to note that the decrease in cohesiveness brought about by formol treatment coincides with histological and histochemical changes comparable to those resulting from storage. Photographs showing the appearance of the cooked formol-treated sample and control are given in Fig. 30. Residual solids from cooking were lesser in treated rice than in control. Formol effects were comparable to those of a five months storage at $\pm 35^{\circ}\text{C}$ (see Table XXIV); residual solids decreased 50%. Water absorption remained practically unchanged. As mentioned above, this was in contrast with the increase brought about by aging. Nevertheless, the volume of the cooked treated rice was larger than that of the control.

The effects of formol treatment on rice composition (Table LVIII) compare fairly well with those of storage. Total N remained practically constant, whereas total alkali soluble, alpha-amino nitrogen liberated after trypsin hydrolysis and N index decreased significantly. Data for the outer layer confirmed the trends of changes. It is of interest to point out that amylose content did not change.

Formol treatment and storage of rice were also found to have parallel effects on the resistance of the kernel to abrasion. Milling during 120 minutes, at the laboratory abrasion mill (see pg. 20), of formol treated rice and of its control resulted in removal of 8.0% and 9.4% outer layer flour respectively. This difference is highly significant at $P=0.001$. Similar effects were determined in rice by storage (Fig. 31): storage brings about a significant hardening of the kernel. (This might be of interest in connection with the preparation of high protein flours by deepmilling).

A different prove of rice hardening during storage was presented previously by Desikachar and Subrahmanyam (101). They reported: "Testing the hardness of new and old rice (in the same variety and dried to the same moisture content) in a stiffness or flatorush testing apparatus (supplied by Gaydon & Co., England, and used in testing of packaging materials) gave indications that the old stored grain was harder than the new grain".

From the results reported on the parallel effects of formol treatment and of storage upon milled rice, it appears that changes in organoleptic characteristics of the cooked kernel as well as those in physical and physicochemical properties of the raw kernel are largely, if not mainly, due to a change in the chemical nature of the protein material. Furthermore, the obtained data lend some support to the view that the protein modification responsible for changes in rice properties might well be an inter-or intramolecular cross-linking formed by a protein-carbonyl compounds interaction.

Table LVII.- Effects of carbonyl compounds on composition and properties of milled rice.

Characteristics	Entire kernel		
	Untreated rice	Treated rice	Storage changes untreated rice
Cohesiveness	7.0	9	increase
Size {	width (mm)	3.5	3.8
	thickness (mm)	2.7	2.8
	length (mm)	9.5	10.9
Water absorption (1)	74	73	increase
Solids from cooking (2)	11.0	5.4	decrease
Total N (%)	1.64	1.70	no change
Alkali soluble proteins (%)	8.40	2.16	decrease
N index	230	25	decrease
Alpha-amino N (4)	117	50	decrease
Amylose (%)	24.3	23.2	no change (3)
Outer layer (6)			
Total N (%)	3.10	3.15	no change
Alkali soluble proteins (%)	17.30	3.25	decrease
Alpha-amino N (4)	419	178	decrease
Amylose (%)	17.70	17.80	no change
Trypsin action			
Cohesiveness	2.4	7.1	increase
Susceptibility to trypsin (5)	0.15	0.05	decrease

(1) g H₂O/100 g cooked rice. (2) g/100 g rice. (3) See pgs. 63 and 98. (4) mg N liberated after trypsin hydrolysis/100 g rice flour, dry basis. (5) g N rendered soluble by trypsin/100 g rice. (6) \cong 8% of the kernel weight.



Fig. 30.- Photographs showing the effects of formal treatment of raw rice on the appearance of cooked kernels. Left: untreated; right: treated.

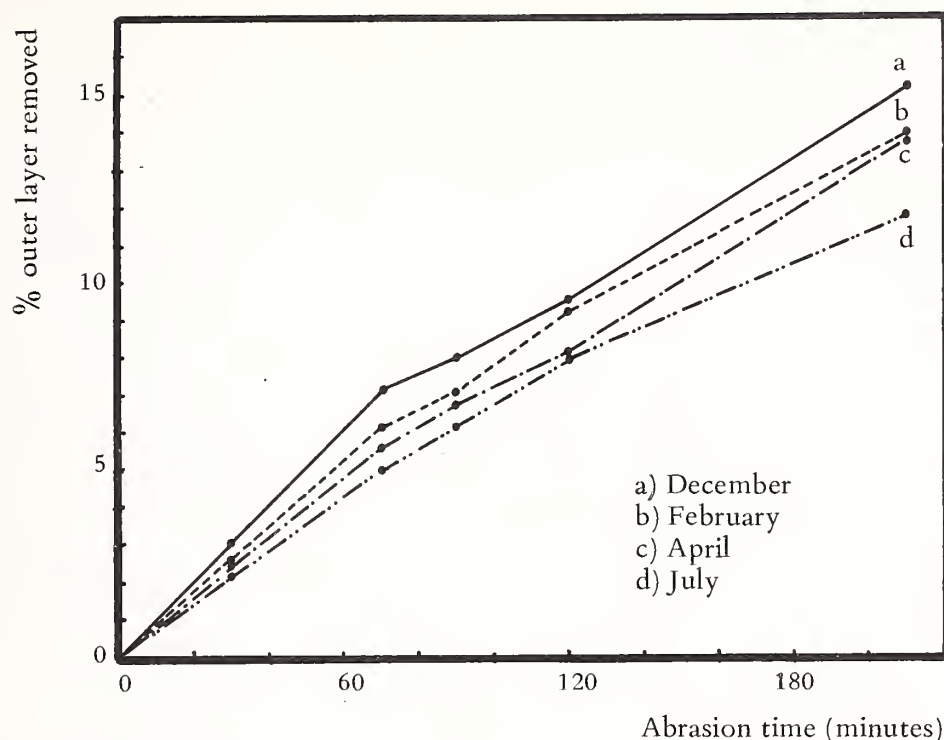


Fig. 31.- Effects of storage on resistance of milled rice to abrasive milling (Storage conditions: air-tight storage; moisture content, 13.2 %; temperature, 35°C).

2. Studies on the influence of lipids upon changes in composition and properties of milled rice during aging.

2a. Effects of defatting prior to storage on composition and properties of aged rice. Defatting did not bring about significant changes in composition and properties of new rice -lipids apart- (Table LVIII). Therefore, differences between aged non defatted and aged defatted samples could be ascribed to storage.

Storage resulted in some differences in organoleptic properties of cooked rice between defatted and non defatted samples (Table LVIII). Remarkable differences were only noticed in alpha-amino N and amylogram characteristics. Storage changes were somewhat smaller in the defatted sample than in the control, as it was the case with cohesiveness and off-flavors. In general, differences were small, being difficult to withdraw clear conclusions. Unfortunately, defatting was not complete; need for using entire kernels made difficult to exhaustively extract the lipid material.

2b. Effects of defatting prior to curing on composition and properties of cured rice. Milled rice and partially defatted milled rice, cured as described in the Experimental Part, were characterised by taste-panel test and physical and chemical analysis. Results from two experiments are given in Tables LIX and LX.

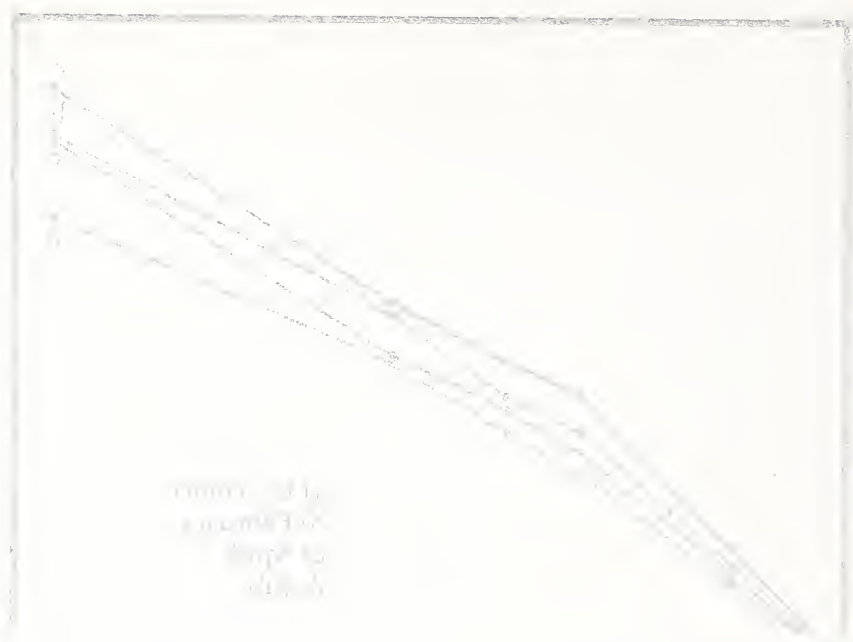


Table LVIII: - Effects of defatting prior to storage on composition and properties of aged rice.

Characteristics	Non-defatted rice		Defatted rice	
	New	Old (1)	New	Old(1)
<u>Organoleptic</u>				
Cohesiveness	6.3	7.5	6.4	6.9
Off-flavors	9	7	9	9
Overall acceptability	5.6	5.4	5.4	6.0
<u>Chemical</u>				
Fat content (2)	0.60	0.60	0.12	0.12
Peroxide value (3)	5.3	8.8	0	0
Total CO content {	30.4	25.0	30.6	25.0
	0.18	0.15	0.04	0.03
Iodine value (6)	113.0	103.8	111.3	107.8
N content (7)	1.20	1.23	1.31	1.35
Alpha-amino N (8)	123	81	123	77
Available lysine {	264	264	260	253
	3.70	3.60	3.33	3.14
<u>Rice flour amylogram</u>				
Gelatinization temperature (°C)	85.5	87.0	-	84.5
Peak viscosity (B.U.)	625	800	-	745
Breakdown (B.U.)	165	250	-	210
Viscosity at 50°C (B.U.)	970	1170	-	1060
Set back (11)	505	630	-	525

(1) Air-tight storage; + 25°C; M.C.: non-defatted 14.10%, defatted 13.84%; milling degree, 6.9%; storage time, five months. (2) g/100 g rice, d.b. (3) meq/Kg. fat. (4) μ mol CO/g fat. (5) μ mol CO/g rice, d.b. (6) g I₂/100 g fat. (7) g N/100 g rice, d.b. (8) mg alpha-amino N/100 g rice, d.b. (9) mg/100 g rice, d.b. (10) g/100 g protein. (11) Increase in viscosity during cooling up to 50°C.

Organoleptic data showed no difference in cohesiveness between cured undefatted and cured defatted samples. Color of both uncooked and cooked samples was darker in the undefatted rice and texture was somewhat more tender in the defatted rice. This -and off odors in lesser extent- seems to explain the small difference in overall acceptability. Physical and chemical data did not reveal significant differences between cured undefatted and cured defatted samples -lipids apart. Histochemical work did not show clear differences (Fig. 32). Only, amylogram characteristics (Table LX) did it: in general, changes were smaller in undefatted rice.

Partial defatting previous curing appeared to have negligible influence on composition (lipids apart) and properties of cured rice. Residual lipids might perhaps be responsible for the lack of significant differences. Curing treatment (18 hrs at 78°C) was drastic and the 0.1% fat content of partially defatted rice could become affected. In fact, CO increased during curing.

Table LIX.- Effects of defatting prior to curing on composition and properties of cured milled rice. (Experiment I).

Characteristics	Non-defatted rice		Ethyl ether extracted rice	
	Uncured (1)	Cured	Uncured (1)	Cured
<u>Organoleptic</u>				
Cohesiveness	6.3	8.2	6.4	8.1
Off-flavors	9	6	9	6
Overall acceptability	5.6	3.7	5.4	4.5
<u>Chemical</u>				
Fat content (2)	0.60	0.56	0.12	-
Peroxide value (3)	5.3	19.5	0	-
Total CO content { (4)	30.4	38.8	30.6	-
(5)	0.18	0.21	0.04	0.09
Iodine value (6)	113	117	111	-
N content (7)	1.20	1.23	1.31	1.25
Alpha-amino N (8)	123	40	123	43
Available lysine { (9)	264	235	260	245
(10)	3.70	3.22	3.33	3.27
Blue test (11)	53.8	54.4	53.9	54.0
Alkali test { Spreading	6.1	6.5	6.1	6.3
{ Clearing	5.1	5.5	5.2	5.3
N index	198	131	189	143
<u>Rice flour amylogram</u>				
Gelatinization temperature (°C)	85.5	80.5	-	-
Viscosity at 94°C (B.U.)	480	860	-	-
Peak viscosity (B.U.)	625	1185	-	-
Breakdown (B.U.)	165	120	-	-
Viscosity at 50°C (B.U.)	970	1860	-	-
Set back (12)	505	795	-	-
<u>Rice starch</u>				
Swelling power	16.3	16.7	-	-
Solubility (%)	21.6	22.4	-	-
<u>Rice starch amylogram (13)</u>				
Gelatinization temperature (°C)	70.5	71.0	-	-
Viscosity at 95°C (B.U.)	505	500	-	-
Viscosity at 50°C (B.U.)	1270	1095	-	-
Set back (12)	400	340	-	-

(1) Data taken from table LVIII. (2) g/100 g rice, d.b. (3) meq/Kg. fat. (4) μ mol CO/g fat. (5) μ mol CO/g rice, d.b. (6) g I₂/100 g fat. (7) g/100 g rice, d.b. (8) mg alpha-amino N/100 g rice, d.b. (9) mg/100 g rice, d.b. (10) g/100 g protein. (11) % transmission. (12) Increase in viscosity during cooling up to 50°C. (13) Amylograms presented no peak viscosity.

Table LX.- Effects of defatting prior to curing on composition and properties of cured milled rice. (Experiment II).

Characteristics	Non-defatted rice		Ethyl ether defatted rice		Methanol extracted ethyl ether defatted rice	
	Uncured	Cured	Uncured	Cured	Uncured	Cured
<u>Organoleptic</u>						
Cohesiveness	6.5	8.5	6.5	8.3	6.1	6.2
Off-flavors	9	6	9	7	9	9
Overall acceptability	5.5	4.1	5.5	4.5	2.1	2.3
<u>Chemical</u>						
Fat content (1)	0.71	0.72	0.11	0.10	0	-
Total CO {	(2) 29.5	23.6	63	100	-	-
	(3) 0.21	0.17	0.07	0.10	-	-
Alpha-amino N (4)	81	59	84	56	42	38
Blue test (5)	-	-	-	-	45.3	45.8
<u>Rice flour amylogram</u>						
Gelatinization temperature (°C)	87.0	83.0	86.0	82.0	77.0	76.5
Viscosity at 94°C (B.U.)	525	700	525	690	740	800
Peak viscosity (B.U.)	635	1000	625	925	830	920
Breakdown (B.U.)	170	245	150	180	150	140
Viscosity at 50°C (B.U.)	960	1440	945	1360	1160	1250
Set back (6)	495	685	465	610	480	470
<u>Rice starch amylogram (7)</u>						
Gelatinization temperature (°C)	69.0	69.0	69.0	70.0	-	-
Viscosity at 95°C (B.U.)	560	500	560	520	-	-
Viscosity at 50°C (B.U.)	1180	1200	1390	1250	-	-
Set back (6)	400	380	490	400	-	-

(1) g/100 g rice, d.b. (2) μ mol CO/g fat. (3) μ mol CO/g rice, d.b. (4) mg alpha-amino N/100 g rice, d.b. (5) % transmission. (6) Increase in viscosity during cooling up to 50°C. (7) Amylograms presented no peak viscosity.

It is thought that introduction of a few carbonyl groups might be effective in modifying rice properties, and this could take place both in undefatted and partially defatted rices.

Curing under conditions tested brought about significant changes in cohesiveness, volume, texture and appearance of cooked kernels. Starchy material did not appear to be associated with these changes. The swelling power and solubility (Table LIX), and amylogram's characteristics (Tables LIX and LX) of starches isolated from uncured and cured rices were similar. Since mild alkaline treatment of rice during starch isolation could not destroy crosslinks in starch, acetal formation (resulting from a carbonyl-starch interaction) is not probably

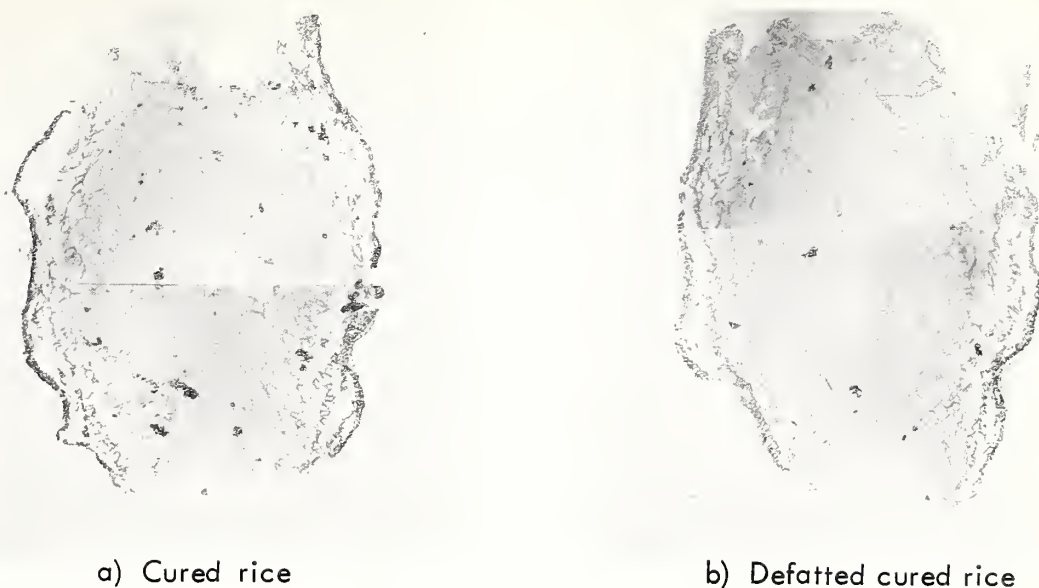


Fig. 32. - Effects of defatting prior to curing on microscopical structure of cured milled rice. Photomicrographs of transections of cooked cured rice. Stained with erythrosin B. Magnification $\times 20$.

formed during curing. On the other hand, protein material changed significantly -N index and alpha-amino N liberated after trypsin hydrolysis and, in lesser amount, available lysine (Tables LIX and LX).

In contrast with this, large differences in amylogram's characteristics were found between uncured and cured rices (Tables LIX and LX). It appears therefore that non starchy constituents are the factors responsible for differences in the pasting characteristics of flour due to curing.

The differences in amylogram's characteristics between uncured and cured undefatted rices were not in complete agreement with data reported previously by other authors (267) (276)(403). It appears that the effects of curing are dependent on extent of treatment. Notwithstanding, increased tendency to retrogradation with curing is a common feature. In this connection, it may be of interest to quote the comments by Normand et al. (267): "The X-ray patterns of the heat-treated and control samples were indistinguishable..." "The X-ray patterns of all samples tested were of the A spectrum that is typical for native granular rice starches. There was no evidence of any B spectrum which would have indicated possible retrogradation". This seems to be in agreement with the data here obtained for isolated starches.

Study of properties of exhaustively MeOH-extracted ethyl-ether-defatted rice added further support to the view that lipids have an important role in curing (and storage) changes. Ethyl ether, methanol extracted rice (x) did not undergo significant change during curing (Table LX). Protein changes, as measured through alpha-amino N liberated after trypsin hydrolysis were negligible in absence of lipids.

(x) Residual lipids content was non measurable.

Table LXI. - Effects of addition of methyl linoleate prior to storage on composition and properties of aged defatted milled rice.

Characteristics	Experiment I		Experiment II	
	Control(1)	Problem(2)	Control(3)	Problem(4)
<u>Organoleptic</u>				
Cohesiveness	6.2	8.2	6.5	8.5
Off-flavors	-	-	6	1
Overall acceptability	5.1	2.2	4	1
<u>Chemical</u>				
Fat content (5)	-	-	0.89	0.83
Total CO content {	-	-	34	641
	0.11	0.43	0.3	5.7
N content (5)	1.25	1.23	-	-
Alpha-amino N (8)	107	70	93	67
N index	198	180	-	-
Alkali test {	4.6	4.3	-	-
	3.5	3.2	-	-
Blue test (9)	56.1	56.3	-	-
<u>Rice flour amylogram</u>				
Gelatinization temperature (°C)	-	-	86.5	84.5
Viscosity at 94°C (B.U.)	-	-	440	830
Peak viscosity (B.U.)	-	-	600	1000
Breakdown (B.U.)	-	-	110	300
Viscosity at 50°C (B.U.)	-	-	1070	1380
Set back (10)	-	-	580	680
<u>Rice starch amylogram (11)</u>				
Gelatinization temperature (°C)	-	-	69.5	69.0
Viscosity at 95°C (B.U.)	-	-	560	530
Viscosity at 50°C (B.U.)	-	-	1270	1230
Set back (10)	-	-	500	350

(1) Defatted milled rice with methyl linoleate added, stored under N₂ at +5°C, during seven weeks. (2) Id. stored under O₂ at +37°C. (3) Id. under N₂ at +5°C, during five weeks. (4) Id., under O₂ at 37°C. (5) g/100 g rice, d.b. (6) μ mol CO/g fat. (7) μ mol CO/g rice, d.b. (8) mg alpha-amino N/100 g rice, d.b. (9) % transmission. (10) Increase in viscosity during cooling up to 50°C. (11) Amylograms presented no peak viscosity.

Table LXIII.- Changes in fatty acid composition during storage of defatted milled rice with methyl linoleate added.

Fatty acid (%)	Control (1)		Problem (2)	
	Before storage	After 30 days storage	After 30 days storage	After 45 days storage
Palmitic acid	4.1	4.7	7.6	9.6
Stearic acid	-	0.3	1.5	5.4
Oleic acid	22.1	25.6	35.7	42.9
Linoleic acid	71.0	65.3	52.6	39.4
Others	2.8	4.1	2.6	2.7

(1) Ethyl ether defatted milled rice with methyl linoleate added stored under N_2 at $+5^\circ C$.

(2) Id., stored under O_2 at $+37^\circ C$.

It should be mentioned that methanol extraction brought about remarkable changes in the eating characteristics of partially defatted rice (Table LX). Whether these changes are due to extraction of "simple lipids" (fatty acids extractable with polar solvents), "complex lipids" (lipoproteins), dehydration, or protein alteration was not investigated, but it is believed it would be worthy of further study.

2c. Effects of addition of methyl linoleate prior to storage on composition and properties of aged defatted milled rice. Samples of ethyl ether-extracted milled rice with methyl linoleate added were stored: a) under N_2 at $+5^\circ C$ - "control" - and b) under O_2 at $+37^\circ C$ - "problem". The samples were characterised after seven weeks' storage. Results from two experiments are given in Tables LXI and LXII. Deterioration of methyl linoleate in "problem" sample was pronounced as expected (Table LXII). Rancid flavors were strong. Concomitant changes in cohesiveness and overall acceptability were clearly noticed by the panel. Cooked kernels of "problem" sample were lesser sticky and drier than those of control. Overall acceptability was lower because of off-flavors. There were no differences in fat content, N content, alkali test values and blue test value between "control" and "problem". Alpha-amino N and N index were lower and CO content higher in "problem" than in "control". Amylograms' characteristics of rice flour pastes also were different. In contrast with this, amylogram's characteristics of starch pastes were identical.

From the studies on the influence of lipid upon storage changes in composition and properties of milled rice the following conclusions can be withdrawn: a) Proteins are chemically modified during storage. b) Deterioration products of lipid oxidation appear to be responsible for such change, forming crosslinked proteins through a carbonyl-protein interaction mechanism. c) Quality changes in rice during storage are closely associated with protein changes. There is considerable evidence in support of the view that protein is a major factor determining quality changes of rice. d) On the basis of obtained data, starch plays a minor role in these changes.

GENERAL CONCLUSIONS

1. Bibliography on composition, storage changes and quality of rice is voluminous, but tremendously dissiminated, and even difficultly attainable in a large part. A few individual efforts for comprehensive surveys have been done, but in spite of their high quality work, the problem still remains. An international program directed to compile and criticise published information is urgently needed. It would be the best fundamental basis for a rapid development of the chemistry and technology of rice.
2. The average chemical composition data, conventionally used up to now, to evaluate rice provide an inaccurate estimation of the chemical nature of the cereal; this may be often misleading.
3. Quantitative and qualitative differences between successive layers of the rice kernel have been found, they showing that the actual chemical nature of rice is different from and more complicated than previously thought. This knowledge opens new possibilities to the chemistry and utilization of rice.
4. The outer layer of the kernel, with an unusual chemical composition, is of basic interest in studying the reactivity and properties of rice.
5. The uniformity and extent of milling have an important influence on the chemical composition of outer layer, and therefore, they should not be undervaluated.
6. The pattern of distribution of chemical constituents is common for all varieties investigated. Varietal differences in the concentration gradient occur, thereby it is difficult to establish a relationship between the chemical composition of the outer layer and that of the entire kernel.
7. Storage results in significant changes in the chemical composition and properties of milled rice. They should be of greater concern to consumers, particularly to the processor.
8. Storage does not necessarily imply deterioration. Adequate storage brings about desirable changes in the characteristics and properties of rice. Undesirable or desirable effects resulting from the chemical reactions involved in aging depend on environmental conditions, length of time and on the rice itself. Undermilling, and high moisture content and temperature accelerate the changes.
9. Storage changes occur at a more rapid rate in the outer layer than within the nucleus of the kernel. During milling, abrasion destroys the cell organization of the kernel surface, which has an unusual reactive composition; the natural stability is lost and the opportunity of the constituents to react with one another is highly increased. In addition, other factors such as microflora -concentrated on the surface- and the direct contact with environment contribute to more accelerated reactions. Changes in outer layer are responsible for a major part of changes in rice kernel properties.

10. Separate consideration of the outermost layer of the kernel is recommended to follow the effects of storage on rice; it affords a more actual and sensitive means than the entire kernel.
11. Histochemistry has proved to be a helpful tool to establish a reasoned connection between chemical and organoleptic data. This, and the study of the effects of physical, chemical or biochemical treatments on rice offered much opportunity for a rapid progress in the study of the factors determining the behaviour of rice during cooking and the properties of the cooked kernel.
12. No single factor appears to be an absolute measure of rice quality. The composition of the outer layer of the rice kernel largely governs cooking and eating quality. Proteins in this layer appear to be the predominant factor. The amount of proteins in outer layer may be a useful criterion to evaluate rice, but it is not always sufficient. Proteins distribution and chemical nature - this, still insufficiently known - influence the rice behaviour.
13. The possibility of a procedure for measuring the cooking and eating quality of rice, involving quantity, distribution, and chemical nature of proteins in outer layer, has not been sufficiently investigated. More knowledge on the nature and physicochemical properties of proteins is needed.
14. Changes in rice properties during normal and accelerated storage have been found to be mainly due to modification of the chemical nature of proteins. Results suggest an inter- or intra- molecular crosslinking resulting from an interaction of proteins with breakdown products of lipid oxidation - carbonyl compounds.

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- a) Effects of storage on physico-chemical characteristics and cooking and eating quality of milled rice.
- b) Formation of CO_2 during storage of milled rice.
- c) Studies on the microscopical structure of the cooked rice kernel and of its relation with proteins, starch and organoleptic characteristics.
- d) Studies on the occurrence of an interaction of proteins with breakdown products of lipid oxidation during storage of milled rice.





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